Renewable and Sustainable Energy Reviews 27 (2013) 622–653

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—A review

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ARTICLE INFO

Article history:
Received 8 June 2012
Received in revised form
22 May 2013
Accepted 26 May 2013
Available online 7 August 2013

Keywords:
Microlgae
Biofuel
Digester effluent
Photobioreactor
Transesterification
CO_2 capture
Wastewater treatment

ABSTRACT

Algae have recently received growing attention given its prospects as a source of renewable energy and its potential for CO_2 capture. Algae culture is of increasing value given that: (i) algae can be cultivated on non-agricultural land using wastewater, (ii) algae can provide a high yield on a per unit of light irradiated area, (iii) algae growth requires CO_2 and nutrients that can be obtained from wastewater and fossil fuel combustion and (iv) algae contains high oil and starch making possible the production of high quality biodiesel. Thus, algae culture can contribute to CO_2 fixation, wastewater treatment and can be a source of bioenergy. This article presents a critical review, focusing on various microalgae species that consume CO_2 and nutrients from wastewater, and provide high quality biofuel. In this respect, a number of relevant topics are discussed in this review: (a) the media for algae culture, (b) the photobioreactor, (c) the associated wastewater treatment processes, (d) the CO_2 capture mechanism and (e) microalgal harvesting. This review also considers various aspects of the biomass processing such as (a) lipid extraction, (b) thermodynamics of the produced biomass conversion, (c) biomass gasification, (d) biodiesel production, (e) catalysts, (f) reaction pathways/mechanisms and (g) reaction kinetics.

Contents

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1364-0321/$ - see front matter
http://dx.doi.org/10.1016/j.rser.2013.05.063

1. Introduction

Global energy demand is drastically increasing due to the growing world population and the improvement of the quality of human life. Conventionally, fossil fuels have been the main source of worldwide energy. However, the continued use of fossil fuels is now commonly considered as unsustainable. This leads to both depleting non-renewable energy reserves and many environmental issues related to fossil fuel combustion.

Combustion of fossil fuels releases large amounts of CO_2 into the atmosphere. This is a major source of greenhouse gases, contributing to the global warming [1]. Statistics indicate that fossil fuel based power generation contributes to about one-third of the total CO_2 released from fuel combustion [2]. A number of international accords such the one of the Kyoto Protocol in 1997 set the stage for worldwide efforts to reduce CO_2 emissions. In order to achieve these ambitious targets, it is required to mitigate the footprints of energy generation using multi-faceted approaches that include nuclear, solar, hydrogen, wind, geothermal, fossil fuels with carbon sequestration/use, and biofuels. Biofuels derived from bio-resources can be classified using the phase they are produced in, by solid, liquid and gas [3]. These fuels are renewable and designated as green energy sources, given their net zero CO_2 emission.

Currently, countries including USA and Brazil produce biodiesel and bioethanol employing human food chain raw materials such as corn, sugarcane, sugar beet, sorghum and wheat. However, the food chain based biofuel industries are receiving increased criticism. This is due to the competing demands of the same sources for human consumption as food. As an alternative, there are other types of biofuels originating from non-food feedstocks such as agricultural wastes, municipal wastes, microalgae and other microbial sources [3]. These alternative feedstocks are more attractive and more acceptable. Their direct or after processing utilization leads to products unsuitable for human consumption. The use of these bio-wastes also help with disposal issues of waste and improve CO_2emission reduction efforts [4]. For example, the cultivation of microalgae consumes CO_2 that can be traced back to fossil fuel combustion processes [5,6].

Nowadays, the use of microorganisms and their metabolic products by humans is one of the most significant fields of biotechnology. Biotechnology deals with the use of microorganisms for the conversion of certain substances into others of greater added value of relevance is the possibility of using a wide variety of substrates for viable products and sub-products, which enables a rational and balanced use of natural resources [7].

Algae may range from single-cell microalgae species to complex multi-cellular giant bladder kelp species. There exists a diverse aquatic family of photosynthetic eukaryotes [8]. Microalgae are microscopic organisms that typically grow suspended in water and are driven by the same photosynthetic process adopted by higher plants. However, unlike higher plants, algae do not require a vascular system to transport nutrients. In addition, and given that every cell is photoautotrophic, they can directly absorb dissolved nutrients [9]. These eukaryotics are sunlight-driven cell factories that can convert carbon dioxide into raw materials for biofuels, animal food chemical feedstocks and high-value bioactive products [10]. Microalgae are amenable to genetic engineering and exploitation in mass cultures for both biomass production and carbon sequestration [11]. For these reasons, microalgae have

Notations

PAR	photosynthetically active radiation
ATP	adenosine triphosphate
ADP	adenosine diphosphate
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
PSI	photosystem I
PSII	photosystem II
PC	plastocyanin
Fd	ferredoxin
Cyt b6f	cytochrome b6f complex
FNR	ferredoxin-NADP reductase
LHC	light harvesting complex
Ru5BP	ribulose 1,5-bisphosphate
PGA	3-phosphoglycerate
PGAL	3-phosphoglyceraldehyde
GP	glycerate 3-phosphate
G3P	glyceraldehyde 3-phosphate
G6P	glucose-6-phosphate
RuBisCO	ribulose bisphosphate carboxylase/oxygenase
1,3-BPGA	1,3 bis-phosphoglycerate
3-PGA	3-phosphoglycerate
PEP	phosphoenolpyruvate
R_{CO_2}	CO_2 fixation rate (g CO_2 m^{-3} h^{-1})
μ_L	volumetric growth rate (g dry weight/m^3 h)
M_{CO_2}	molecular weights of CO_2
M_C	molecular weights of elemental carbon
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
COD	chemical oxygen demand
TKN	total Kjehldahl nitrogen (mg/L)
BOD	biological oxygen demand
NTU	nephelometric turbidity units
TSS	total suspended solids
DO	dissolved oxygen
SS	suspended solids
TOC	total organic compound
TP	total phosphorus
HRT	hydraulic residence time
PFD	photon flux density
OCD	optimal cell density (g/L)
O.D.	optical density (g/L)
μ	specific growth rate (T^{-1})
g	cell generation period (T)
X	biomass concentration(ML^{-3})
V	culture volume (L^3)
A	culture area (L^2)
P	per unit areal yield (ML^{-2} T^{-1})
S	limiting nutrient
K_S	half saturation coefficient (mg/L)
A	Arrhenius rate constant (d^{-1})
E	activation energy (Cal/mole)
R	universal gas constant (cal K/mole)
T	temperature (K)
CTAB	*N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide
SDS	sodium dodecylsulfate
FA	fatty acid
FFA	free fatty acid
GC	gas chromatography
GC-FID	gas chromatography-flame ionization detection
GC–MS	gas chromatography–mass spectrometry
HPLC	high performance liquid chromatography
FAME	methyl esters of the fatty acids

found numerous beneficial applications including animal food fertilizer, food stocks for pharmaceutical industry, pollution control, water treatment, dyes, agar manufacturing and production of a second generation biofuel [12]. Specifically, their ability to capture CO_2 makes microalgae cultivation an attractive alternative for CO_2 sequestration. This capture of CO_2that can be traced to fossil fuel power plant gas effluents contribute to the reduction of green house gas emissions [4].

Interestingly, unicellular microalgae can grow in waste water containing high nutrient concentrations, helping with water treatment. The treatment of livestock effluents from dairy farms with microalgae has also received increasing attention. This is due to the fact that the uncontrolled nutrient discharges coming with dairy waste may cause severe problems in aquatic ecosystems and pollute strategic groundwater resources [13]. Conventional aerobic treatment methods, such as activated sludge processes, involve oxygen supply which are energy demanding. These processes also entail the impractical recycling of valuable nutrients contained in dairy effluents [14,15]. Anaerobic digestion is generally applied in a variety off-forms and scales to stabilize the organic matter in wastewater. These processes can be effective for organic matter and pathogen reduction. They can generate biogas as well, which can be used as a fuel [15].

However, effluents of anaerobic digestion of dairy wastewaters are characterized by a high ammonia content that must be pretreated before further processing [16]. During storage and usage, digester effluents may lead to large amounts of nitrogen escaping into the atmosphere due to ammonia volatilization [17]. In this respect, environmentally friendly and economically sound manure management in dairy farms is vital to their sustainability and to the minimization of losses of valuable plant nutrients. Microalgae and cyanobacteria offer low cost processes. They can be utilized as bioremediation agents to remove inorganic nutrients from wastewaters and to improve water quality due to their high capacity of nutrient uptake [18]. Microalgae that are produced in the bioremediation of dairy wastes can be further processed into different types of biofuels such as biodiesel, jet fuel, biogas and biohydrogen [19–25].

Fig. 1 displays a simplified process flow diagram of an integrated microalgae culture-wastewater treatment-biofuel production process. It shows that microalgae grow using nutrients of the wastewater and CO_2 from a combustion process. Finally, the cultivated microalgae can be employed to produce energy and food supplements for humans and animals. Thus, one can notice that commercial scale CO_2 capture with wastewater treatment and biofuel production using microalgae culturing entails a holistic approach. This approach requires that various process steps will be integrated effectively in the context of a single industrial facility.

Thus, the cultivation of microalgae offers the following three important advantages:

(i) capture of CO_2 emitted from fossil fuel based power generating stations,
(ii) treatment of wastewater,
(iii) production of renewable energy.

Keeping these advantages in mind, research studies are currently being developed to establish processes and technologies for

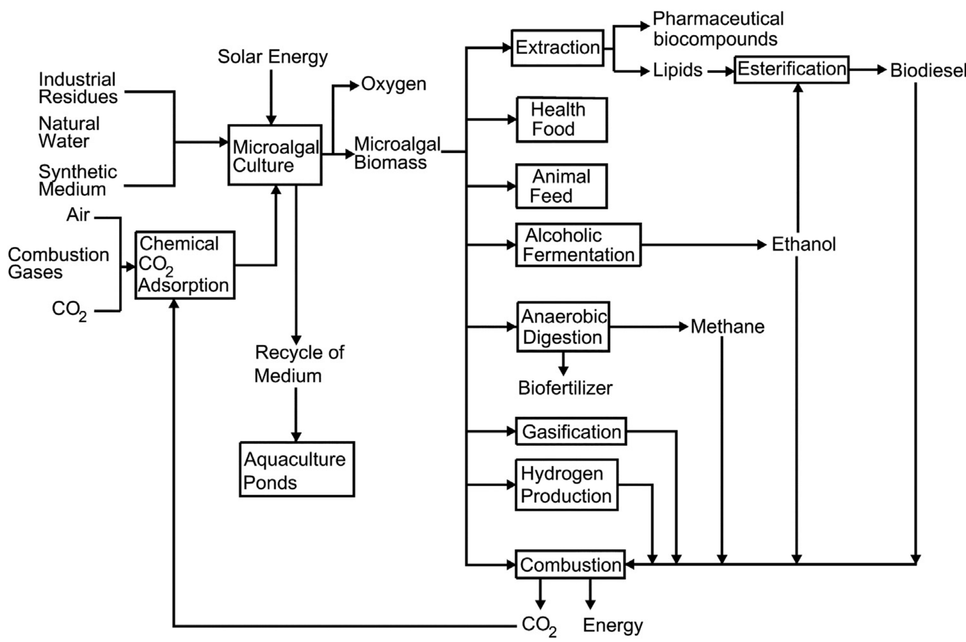


Fig. 1. Simplified process flow diagram envisioned for algae wastewater treatment with CO_2 mitigation and biofuel production (adapted from [7]).

application at the industrial scale. This review article focuses on the current state-of-the-art and recent developments of technologies dealing with microalgae culturing for CO_2 capture, wastewater treatment and biofuel production. A valuable example of the commercial prospects of this technology is the "Solutions4CO2" plant being established in Sarnia, Ontario. This 50,000 square foot plant captures carbon dioxide and combines it with wastewater which is high in nitrogen. The plant effluent is used to grow algae under enhanced conditions. The produced algae can be used to make biopharmaceuticals, like the Omega 3 and biofuels [26].

2. Algae species

As mentioned earlier, algae are a diverse family of photosynthetic eukaryotes species. Most of these species are aquatic. Numerous types of algae accumulate oil and lipids have a density lower than water. This lower density helps with their flotation in ponds and lagoons. Over 36,000 different species of algae are available in the natural ecosystem. Algae species can be classified as red algae, green algae, brown algae, diatoms, blue green algae (prokaryotes) or dinoflagellates. Many of these algae have found various beneficial uses including animal food, fertilizer, pharmaceutical drugs production, pollution control, water treatment, dyes, agar manufacture and as source for bioenergy [27].

In early research initiatives, culture systems of microalgae were investigated in a detailed manner as an alternative to protein sources for human consumption. Recently, the focus has been shifted to the use of microalgae culturing for CO_2 capture and biofuel production. Microalgae can capture emitted $CO_2$from a variety of sources and can convert CO_2 into biomass with the help of sunlight and photosynthesis. Moreover, microalgae produced can be further processed to manufacture biofuels [28]. Microalgae have the ability to grow rapidly. Microalgae also can synthesize and accumulate large amounts (approximately 20–50% of dry weight) of neutral lipid stored in cytosolic lipid bodies. As a result, a successful and economically viable algae based biofuel industry mainly depends on the selection of appropriate algal strains [29,30].

Table 1
General composition of different microalgae in percentage of dry weight basis (adapted from [31]).

Microalgae species	Protein	Carbohydrates	Lipids
Anabaena cylindrica	43–56	25–30	4–7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Spirogyra sp.	6–20	33–64	11–21
Arthrospira maxima	60–71	13–16	6–7
Spirulina platensis	46–63	8–14	4–9
Synechococcus sp.	63	15	11

Table 1 reports the general chemical composition of different microalgal species that were reported in the literature [31]. One can easily notice from this table that most of the algae contain large amount of proteins, dominantly enzymatic proteins or crude proteins. These proteins, mainly amino acids, provide high quality nutrients commonly found in foods and feeds for animals.

To grow the algae species, carbon dioxide, water, nutrients and suitable temperature control are needed. Wastewater contains significant amounts of nutrients that are suitable for algae growth. Table 2 summarizes the algal species that have been studied for the removal of nitrogen and phosphorous containing chemical species such as ammonia, nitrates and phosphates from the wastewaters. As can be seen in this table, *Chlorella vulgaris* and *Phormidium laminosum* are two main types of algae species that have been widely studied. Other species studied includes *Anabaena doliolum*, *Chlorella emersonii*, *Chlamydomonas reinhardtii*, *Scenedesmus bijugatus* and *Scenedesmus obliquus*.

In order to achieve the maximum benefit from microalgae culture, it is essential to pay attention to the selection of adequate species or strains. Microalgae culture consists of a single specific strain precisely selected for producing the desired product and the most beneficial outcome of the culture process. It is also important

to observe that required culture conditions are (a) water media at the adequate pH and temperature, (b) necessary contained nutrients and (c) CO_2 dosed in a controlled manner in presence of sunlight. In order to ensure the proper growth of microalgae, the necessary nutrients must be provided. Nutrients can be classified into the following categories: (i) carbon source from CO_2, (ii) energy source from sunlight, (iii) nitrogen source (e.g. ammonia, nitrates) from wastewater or other culture media, (iv) other minerals from culture media and (v) potentially added vitamins.

3. Microalgae cultivation system-photobioreactor technology

There is in the scientific literature, a wide range of the microalgae cultivation systems reported. These microalgae culture systems differ mainly depending on (a) the cost, (b) the type of desired products, (c) the source of nutrients and (d) the CO_2 capture. The culture systems are generally classified according to their design conditions as "open" or "closed" systems. The "open" systems are outdoor facilities that include ponds, lagoons, deep channels, shallow circulating units and others, while the "closed" systems are vessels or tubes with walls made of transparent materials located in outdoors facilities under sunlight irradiation or indoor facilities under artificial irradiation.

Due to their technical complexity, photobioreactors have been considered for a long time as the antithesis of open ponds technology. It is only recently and as a result of the operational difficulties with open ponds that closed bioreactors are being considered as providing useful methods of algal mass culture. The increasing interest in this technology is leading algal culture towards great developments involving quick technical progress [53]. Generally, sophisticated photobioreactors are more versatile but such reactors are expensive and difficult to operate and control. Some studies suggest, however, that the use of photobioreactors is a more feasible and practical option, especially for the removal of CO_2, and for wastewater treatment applications [6,14,20].

Table 2
Summary of algal species for the removal of nitrogen and phosphorus containing compounds.

Microalgae species	Removal of	References
Anabaena doliolum	Nitrogen and phosphorous	[32]
Chlorella emersonii	Phosphorous	[33]
Chlorella kessleri	Nitrogen and phosphorous	[34]
Chlamydomonas reinhardtii	Nitrogen and phosphorous	[35]
Chlamydomonas reinhardti	Nitrogen and phosphorous	[36]
Chlorella vulgaris	Nitrogen and phosphorous	[37]
Chlorella vulgaris	Nitrogen and phosphorous	[38]
Chlorella vulgaris	Nitrogen and phosphorous	[32]
Chlorella vulgaris	Nitrogen and phosphorous	[39]
Chlorella vulgaris	Nitrogen and phosphorous	[40]
Chlorella vulgaris	Nitrogen and phosphorous	[41]
Dunaliella salina	Nitrogen and phosphorous	[42]
Phormidium laminosum	Nitrogen and phosphorous	[43]
Phormidium laminosum	Phosphorous containing species	[44]
Scenedesmus bijugatus	Nitrogen and phosphorous	[45]
Scenedesmus rubescens	Nitrogen and phosphorous	[38]
Scenedesmus bicellularis	Nitrogen and phosphorous	[46]
Scenedesmus intermedius	Nitrogen and phosphorous	[47]
Scenedesmus obliquus	Nitrogen	[48]
Scenedesmus obliquus	Nitrogen	[49]
Scenedesmus quadricauda	Nitrogen and phosphorous	[50]
Spirulina maxima	Nitrogen and phosphorous	[51,52]

3.1. Open system

Open ponds have long been used for large scale microalgae cultivation given their simple construction and relatively easy operation. Such cultivation systems can be classified as (i) natural water systems such as lakes, lagoon, ponds and (ii) artificial water systems such as artificial pond, tanks and containers. Depending on the applications, different shapes, sizes and types of open systems (agitated, inclined and others) have been investigated [54].

Fig. 2 illustrates various ponds that have been reported in the technical literature. Among them, non-stirred ponds (Fig. 2a and c) are the most economical, being the simpler to manage. Commercial non-stirred ponds are built in natural water ponds with less than half of meter in depth. However, this type of pond is very limited in its applications given that algae species cannot survive under frequently poor growth conditions.

One can record, however, an evolution of open systems, with this being the result of trying to address mixing issues in ponds,

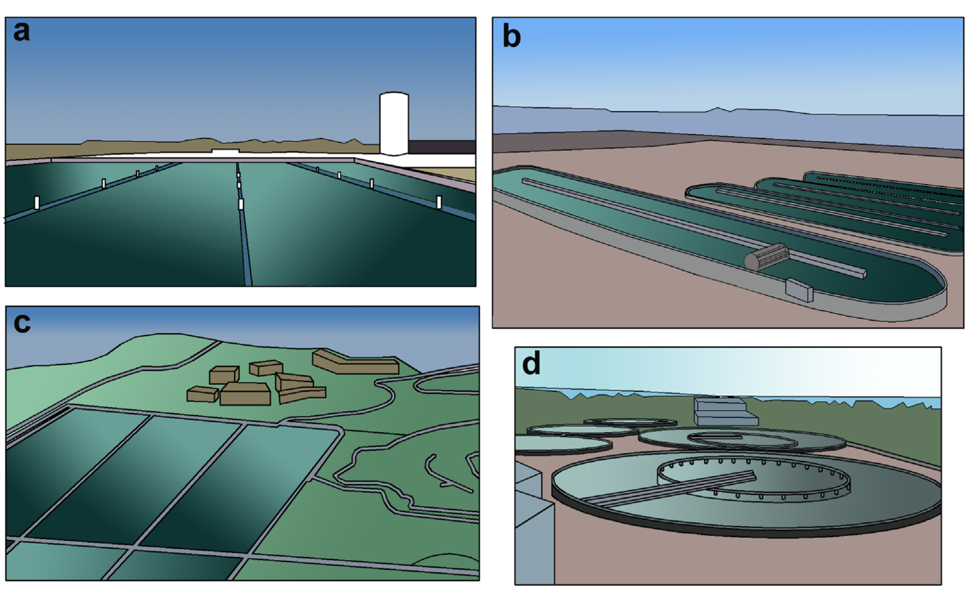


Fig. 2. Open cultivation systems: (a) open unstirred pond for chlorella culture by Solix Biofuel, Colorado, USA. (b) Paddle wheel raceway pond at Carbon Corporation, California, USA. (c) Red algae culture at the San Francisco Bay, California, USA. (d) Circular Ponds in Taiwan (BEAM).

preventing algae sedimentation and enhancing light utilization. Thus, in open ponds, mixing is of great significance with this having an impact on operating costs and productivity. Significant constraints also affect open pond operation. For instance, in high cell-density culture systems, light gradients inside the algae can be seldom prevented, given media, light absorption and cell shading. Furthermore, biomass productivity is not only dependent on the total amount of solar energy penetrating the culture surface, but also on the quantity of energy available at the cell level. This leads to various possible design regimes and operating parameters such as 'light regime' and 'light per cell' as proposed by Richmond [55].

Given all the described matters, the profitable implementation of open culture systems has been limited to very special cases such as production of specialties foods with claimed health benefits [56]. For example, production costs in Japan have been in the range of 10–20 $/kg for *Chlorella* sp. [57]. However, in open ponds, until today, only a few species of microalgae have been found to grow well at a commercial scale.

3.1.1. Unstirred ponds

Unstirred ponds provide an economical and simple way for operating a pond facility. Unstirred ponds are commercially used for some microalgae species cultures such as *Dunaliella salina* [58]. Borowitzka et al. [59] reported that these types of large unstirred open ponds are of simple construction. For example, natural water ponds with less than half a meter in depth can be used. Similar small ponds are shallow water surfaces covered with plastic films [57]. Lee [60] reported that in South-East Asia, over 30 t per year of dried microalgae biomass were harvested from unstirred natural lakes. Unstirred open ponds are, however, limited to growing microorganisms under poor environmental conditions being exposed to the concurrent growth of protozoa, bacteria and viruses [53].

3.1.2. Raceway pond

The most popular open culture system being currently used is the "stirred paddle wheel open pond" or "raceway pond" (Fig. 2b) [54,61]. This type of open pond is usually shallow and commonly in between 15 and 25 cm in depth. Raceway ponds are usually constructed as either a single channel or as groups of channels that are built by joining individual raceways together. In raceway ponds, the productivity of the biomass has been shown to be as high as 60–100 mg dry weight L^{-1} d^{-1} [54,57]. Raceway ponds are mostly used for the commercial culturing of four species of microalgae: *Chlorella* sp., *Spiriluna platensis*, *Hematococcus* sp. and *D. salina* [62].

Different designs of raceway ponds, especially the paddle wheel mixed type, have been used commercially over the last 30 years. The circulation of the cultured media in the raceway pond loop is helped by a paddle. This circulation generates the water velocity required to avoid the deposition of settling cells or the aggregation of cells via flocculation [63]. Many ponds are operated with a liquid velocity of more than 30 cm/s. In many cases, difficulties are encountered with solid deposition in stagnant areas. In raceway ponds, biomass concentrations of up to 1 g dry weight/ L and 60–100 mg dry weight/L d productivities are typically obtained [57,60].

3.1.3. Circular pond

Circular ponds (central pivot) have primarily been used for large-scale cultivation especially in South-East Asia for the culture of *Chlorella* sp. [64]. Circular ponds are the oldest large-scale algae cultivation open ponds. The depth of these ponds is about 25–30 cm. Microalgae are usually grown in concrete circular ponds up to 45 m in diameter, with agitation by a rotating arm (resembling a clock dial with the second rotating hand running around). A 20–30 cm thick layer of inorganic nutrient solution with algae, exposed to sunlight and bubbled by CO_2, is continuously moved by means of paddle wheels (Fig. 2d).

3.1.4. Limitations of open pond systems

The major limitations in open systems include the following: (a) poor light utilization by the cells, (b) significant evaporative losses, (c) limited diffusion of CO_2 from the atmosphere, and (d) large areas of land are required [65]. In addition, contamination is another major problem of open systems with large-scale microalgal production. Unwanted algae, mold, fungi, yeast and bacteria are the common biological contaminants often found in these open systems [57]. To overcome the above limitations, simple plastic covers or green houses over the open ponds have been proposed [66]. Plastic covers also allow extension of the growing period. A permeable plastic cover also facilitates transfer and supply of carbon dioxide and the maintenance of mild temperatures over night hours. It has been reported that the covering of open ponds provides an improvement of biomass productivities [67]. Unfortunately, however, contamination issues still remain unresolved. In addition to that, capital costs, maintenance and overheating make open ponds covered with translucid plastics is impractical with this being especially true for large size units.

3.2. Closed system

Closed systems, mainly known as photobioreactors, can address some of the problems associated with open pond systems. The major advantages of the closed systems are as follows: (a) minimization of water evaporation and (b) reduction of the growth of competitive algal weeds, predators and pathogens that may kill the desired microalgae. It is important to acknowledge that although photobioreactors significantly reduce the growth of competitive algal weeds, they cannot completely eliminate the growth of contaminants [54]. A detailed comparison of different closed photobioreactor systems and their biomass productivities are reported in Table 3.

3.2.1. Tubular photobioreactor

Given the several mentioned disadvantages of open systems, closed systems have been receiving great attention. Several tubular photobioreactors have been studied and developed since the pioneering work of Tamiya et al. [84]. Tubular photobioreactors are made with transparent materials and are placed in outdoors facilities under sunlight irradiation (Fig. 3a). A gas exchange vessel where air, CO_2 and nutrients are added and O_2 is removed is connected to the main reactor [85]. One of the basic characteristics of these cultivation vessel designs is their large surface area per unit volume. This is done to maximize exposure of the microalgae to sunlight. Tube sizes are generally less than 10 cm in diameter to secure sunlight penetration. In a typical tubular microalgae culture system, the medium is circulated through the tubes, where it is exposed to sunlight for photosynthesis. The medium is circulated back to a reservoir with the help of a mechanical pump or an airlift pump. The pump also helps to maintain a highly turbulent flow within the reactor, preventing the algal biomass from settling [1]. A fraction of the algae is usually harvested after it circulates through the solar collection tubes, making the system a continuous operation. Until today, most of the tubular photobioreactors, studied in presence of artificial light have been developed at small/ laboratory scale (0–20 L capacities). There is, in this respect, a limited number of studies reporting data for large-scale closed photobioreactors.

James and Al-Khars [74] studied the growth and the productivity of *Chlorella* and *Nannochloropsis* in a translucent vertical

Table 3
Comparison of different closed photobioreactor systems.

Closed system photobioreactor type	Light source	Capacity (L)	Algal strain	Biomass conc. (g/L)	Biomass conc. (g/m^3 d)	References
Tubular	Artificial	5.5	*Spirulina platesnsis*	0.62		[68]
	Sun	200	*Phaeodactylum tricornutum*	1.19		[69]
	Sun	75	*Phaeodactylum tricornutum*	1.38		[70]
	Sun	10000	*Spirulina*		25	[71]
Airlift	Artificial	3	*Haematococcus pluvialis*	4.09		[72]
	Artificial	170	*Chaetoceros*	0.80		[73]
	Artificial		Nannochloropsis		32.5–95.3	[74]
	Artificial		Chlorella		109–264	[74]
Bubble column	Artificial	170	*Chaetoceceros*	3.31		[73]
	Artificial	1.9	*Phaeoductylum*	-		[75]
	Artificial	4.5	*Monoraphidium*		23	[76]
	Artificial	1.8	Cyanobium sp.	0.071		[77]
	Artificial	3.5	Spirulina	4.13		[78]
	Sun	64	*Monodus*	0.03–0.20		[79]
	Artificial	1.8	*Sc. obliquus*	2.12		[78]
	Artificial	1.8	*Chlorella vulgaris*	1.41		[78]
Flat plate	Articial	3.4	*Dunaliella*	1.5		[80]
	Sun	5	*Phaeodactylum*	1.38		[81]
	Sun	200	*Nannochloropsis*	0.225		[82]
	Sun	440	Nannochloropsis sp.	0.27		[82]
	Sun	25,000			10.2	[68]
Plastic bag	Sun	50	*Tetraselmis*		20–30	[83]

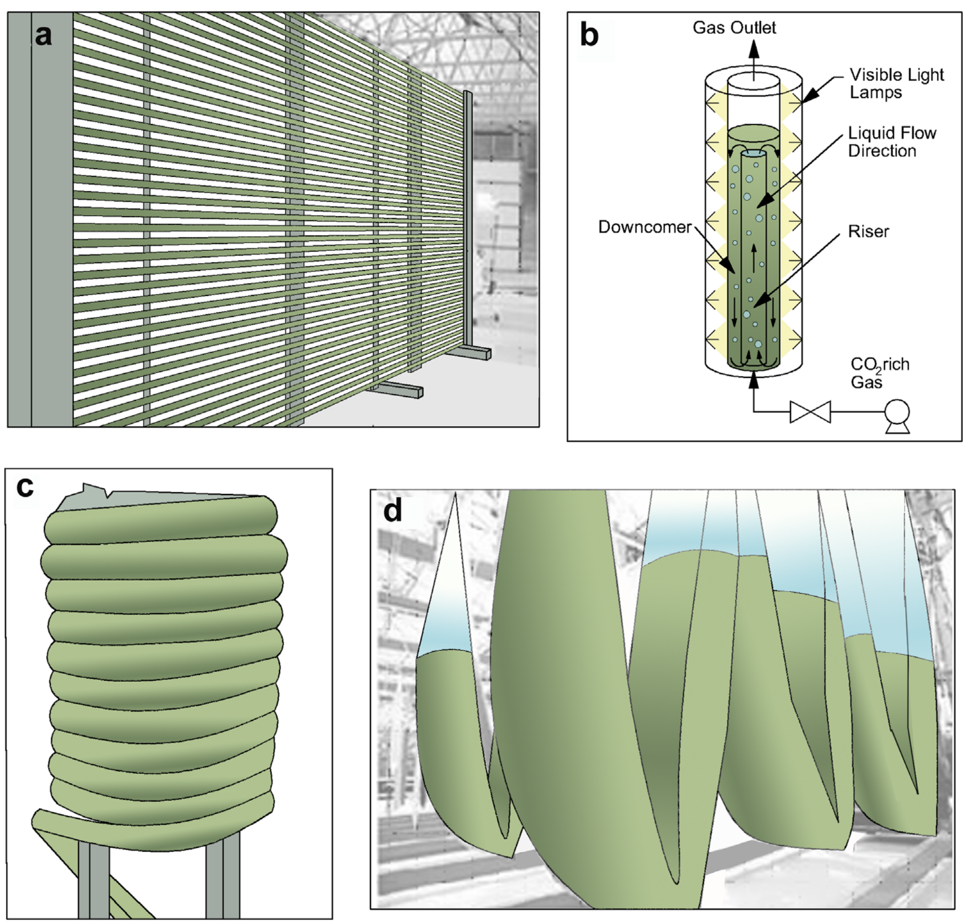


Fig. 3. Closed cultivation system: (a) horizontal tubular photobioreactor at Varion Aqua Solution Ltd., UK. (b) Bubble column air-lift photobioreactor, BBSRC, UK. (c) Helical–tubular photobioreactor. (d) Large-scale plastic bag photobioreactors.

airlift photobioreactor. They obtained productivities between 109 and 264 g/m^3 d for *Nannochlropsis* and between 32.5 and 95.3 g/m^3 d for the Chlorella strain. Miyamoto et al. [76] used a vertical glass tube with 5 cm diameter and 2.3 m height (4.5 L). This is a bubble column unit with good light penetration. Its implementation at full commercial scale-up still seems challenging. The *Monoraphidium* productivity in such reactor has been reported as 23 g/m^3 d.

In these tubular type photobioreactors, tube bundles used as solar irradiation captors are made out of (a) polyvinylchloride (50 mm in diameter) or (b) polycarbonate (32 mm in diameter).

These type of tube bundles were used for algae culturing by Torzillo et al. [71]. The rate of flow in these tubes ranged between 30 and 50 cm/s. From this study, it was concluded that, when the tube diameter is reduced from 50 mm to 32 mm, the optimum population density of *Spirulina platensis* and *Anabaena siamensis* increases, resulting in a higher productivity per culture volume. Torzillo et al. [71] also studied a comparable closed pilot scale photobioreactor (100 m^2, 10 m^3 of culture) initially made of flexible polyethylene tubes (14 cm in diameter) and later of methyl polymethacrylate tubes (13 cm in diameter). The pumping of the culture medium is of the intermittent type in order to maintain an adequate medium flow rate inside the tubular photobioreactor. A diaphragm pump raised the culture media to a feed tank that allowed an intermittent discharge using a siphon into the photobioreactor. At intervals of 4 min., about 350 L of culture suspension were discharged into the photobioreactor, thus moving the culture in the tubes at a rate of 0.26 m/s. This regime of circulation was obtained by adjusting the flow rate of the pump to 4000 L/h. The result was better than with continuous circulation at the same flow rate of the pump. The maximum productivities of *Spirulina* were 25 g/m^2 d.

In some photobioreactors, the tubes are coiled spirals forming helical–tubular photobioreactors (Fig. 3c). Usually, these types of reactors are suitable for the culture of microalgal species in presence of sunlight. Despite this, these systems sometimes require artificial illumination as well as natural light to enhance the microalgae growth. However, the introduction of artificial light adds to production costs, making the helical–tubular bioreactor only adequate for the manufacturing of high-value added products. Another category of closed systems is the airlift photobioreactor. In this reactor, liquid motion is characterized by large circulatory currents in a heterogeneous flow regime [86].

Anderson and Eakin [87] designed a tubular photobioreactor with the capacity to produce polysaccharides using *Porphyridium cruentum* microalgae. The system has a modular design, resembling a solar collector with a photodetector placed at a strategic location for angular adjustment of the glass surface position. The polysaccharide productivities published by the authors ranged from 20 to 25 g/m^2 d.

3.2.2. Plastic bag photobioreactor

There are studies which suggest that microalgae can be produced in transparent polyethylene bags, as shown in Fig. 3d. Generally, these bags are either hung or placed in a cage under the sunlight irradiation. In such arrangements, the algae cultures are mixed with air at the bottom of the bags [54]. Transparent polyethylene sleeves sealed at the bottom in a conical shape which are used to prevent cell settling are also widely used. Using 50 L polyethylene bag cultures operated as turbidostats, Trotta [83] obtained yields of 20–30 g/m^3 d for *Tetraselmis*.

3.2.3. Airlift photobioreactor

In airlift photobioreactors (Figs. 3b and 4), the fluid volume of the vessel is divided into two interconnected zones using a baffle or a draft tube. Liquid movement is characterized by large circulatory currents in the heterogeneous flow regime [86]. Airlift photobioreactors are sometimes difficult to scale-up given their complex flow pattern [88].

Vertical bubble columns and airlift cylinders can attain substantially increased radial movement of fluid, with high cycling of medium between the irradiated and the dark zones. These reactor designs have a low surface/volume ratio. However, they can provide a substantially greater gas hold-up than horizontal reactors with a potentially greater segregated gas–liquid flow [89].

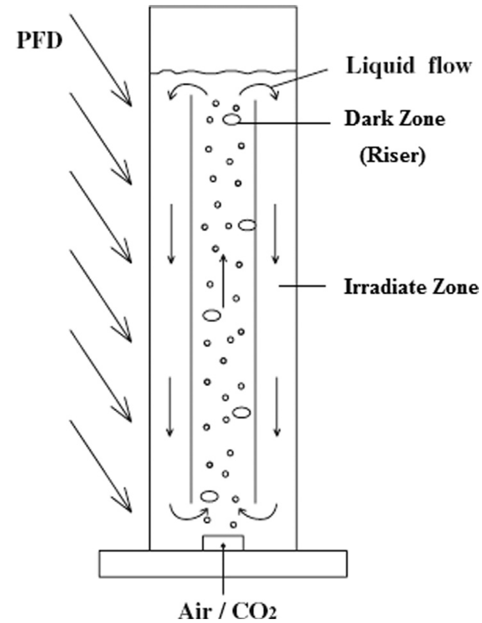


Fig. 4. Air-lift photobioreactor (Adapted from [90]).



Fig. 5. Flat panel photobioreactor (Adapted fromhttp://www.ruhr-unibochum.de/h2design/profile/main.de.html, June 02, 2012).

Other researchers claimed that advantages for these units included (a) high mass transfers, (b) good mixing with low shear stress, (c) low energy consumption, (d) relatively easy to work under sterile conditions, (f) good for the immobilization of algae on moving particles. In these units, cultures suffer less from photoinhibition and photo-oxidation, being subject to cycling effects occurring between lighter and darker zones. Limitations include (a) higher manufacturing and maintenance cost, (b) smaller irradiation per unit surface area, (c) more sophisticated construction materials, (d) higher shear stress on algal cultures, and (e) larger number of units are needed to build a commercial plant given the diameter to height cannot be increased significantly.

3.2.4. Flat plate photobioreactor

Vertical plate photobioreactors (Fig. 5) mixed by air bubbling, seem to be even better than bubble columns in terms of productivity and ease of operation. Flat-plate photobioreactors allow the following: (a) large irradiated surface area, (b) suitable for outdoor

cultures, (c) good for algae immobilization and (d) good biomass productivities. These photobioreactors are relatively cheap and easy to clean [89]. Vertical flat plates can be accommodated in 1000–2000 L volume capacity units that were successfully operated for long time periods (be specific, e.g. several days). Thus, these are fully scalable photobioreactor units [67].

Closed flat panels mixed by bubbling air can potentially achieve high overall ground–aerial productivities in terms of volume cultivation. There are 500 L with 440 L culture volume capacity units with 0.27 g/L d using flat plate glass photobioreactor [82]. Major limitations include (a) difficulty of controlling culture temperature, (b) limited degree of grow that the near wall region, (c) possibility of hydrodynamic stress, and (d) algal strains are subjected to important hydrodynamic stresses [82,89].

Flat plate photobioreactors are recommended for mass production of microalgae in outdoor and indoor culture systems given the following: (a) high irradiation of plate surface, (b) small accumulation of dissolved oxygen while compared to horizontal tubular photobioreactors and (c) convenient modular design for scale-up. Pulz et al. [91] described an optimized large-scale flat plate photobioreactor module of 6000 L. This double layer panel (8 m^2) is laid on the ground. One of the layers is used for the circulation of the culture, while the other is utilized for circulation of the temperature-controlled water. Tredici et al. [92] and Tredici and Materassi [93] developed a vertical alveolar panel (2.2 m^2) based on the same type of material. Flat panels can be used at variable equinoxial orientation with respect to the sun's rays. Mixing and deoxygenation processes of the culture suspension are affected by continuously bubbling air at the bottom of the reactor. Comparable designs with pump and with airlift circulation are also under development [94]. In all cases, high productivities were obtained given the high surface/volume ratio. However, biomass output could be limited by photo-inhibition and temperature control issues.

4. CO_2 capture

Growing industrialization and urbanization are considered to be a major source of CO_2 and it is one of the most important atmospheric pollutants contributing to green house gases. According to the Kyoto Protocol (1997) signed by more than 170 countries, greenhouse gas emissions should be reduced by 5.2% on the basis of the emissions in 1990 [95]. Since then, there are numerous research and development studies that have been undertaken around the world aiming to achieve CO_2 mitigation. Widely studied techniques have included physical, chemical, and biological methods [96–99]. Among these attempts, the biological method using microalgal photosynthesis is believed to be an effective approach to biological CO_2 fixation [95,100]. Microalgal biomass contains approximately 50% carbon by dry weight [101]. All of this carbon is typically derived from carbon dioxide. Producing 100 t of algal biomass fixes roughly 183 t of carbon dioxide. Carbon dioxide must be fed continuously during daylight hours. Control of CO_2 feeding can be helped with pH measurements. pH measurements minimize carbon dioxide losses. Thus, biodiesel production using microalgae culture with carbon dioxide emitted from power plants can reduce the environmental impact of burning fossil fuels [20,96,98,102].

4.1. Photosynthesis

Biological processes provide a promising approach to capture CO_2 in the form of microalgal biomass via photosynthesis. Photosynthesis is the process used by plants to convert water, CO_2 and sunlight with the help of chlorophyll into carbohydrates (Fig. 6).

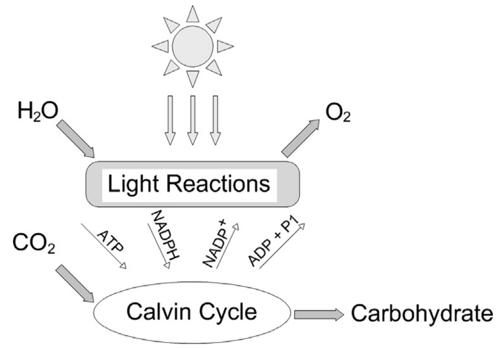


Fig. 6. Schematic diagram of photosynthesis process (Adapted fromhttp://en.wikipedia.org/wiki/Photosynthesis, June 02, 2012).

In microalgae, photosynthesis releases oxygen. This is why it also called "oxygenic photosynthesis". Since CO_2 is converted into lipids and other hydrocarbons in this process, this explains the designation of "*CO_2 fixation process*". In oxygenic photosynthesis, water is the electron donor and after hydrolysis it releases oxygen. The general equation for photosynthesis can be written as

$$H_2O + CO_2 + \text{photons} \rightarrow [CH_2O]_n + O_2$$

The overall reaction is separated into two pathways: (i) light reaction and (ii) dark or light independent reaction. Photosynthesis takes place in chloroplasts which are enclosed by a membrane. This membrane contains an aqueous fluid called stroma. This stroma contains stacks of flattened disks bounded by membrane called thylakoids, which are the active sites of photosynthesis. The sites for photosynthesis are thylakoids membranes which contain protein complexes including pigments (e.g. β-carotene, xanthophylls) that absorb light energy. These pigments are embedded in microalgae in special antenna proteins. This protein is also called "*the light harvesting complex*" [103].

Photosynthetically active radiation, often abbreviated as "PAR", designates the spectral range of solar radiation from 400 to 700 nm that photosynthetic organisms are able to use in the process of photosynthesis. Due to the low absorption capacity of the culture media at 450–650 nm, chlorophyll is able to capture about 30–40% of PAR. Microalgae can optimize the capturing of light by changing the quantity by additional light capturing pigments. The photosynthetically active range of the chlorophyll spectrum lies at and between 680 and 700 nm. The photon energy captured at shorter wavelengths can be transferred to the 680–700 nm longer wave length region. During this photon transfer process from high-energy and shorter wavelength photons to lower energy and longer wavelength photons, significant energy losses occur. As a result, there is a net loss of about 21% of the original light sun energy [104].

4.1.1. Light dependent reaction

The light dependent reaction involves both photochemical and redox reaction steps. In the thylakoids membranes of the chloroplasts, chlorophyll pigments absorbs light energy/photons and release electrons. The overall equation for the light-dependent reactions is [105]

$$2H_2O + 2NADP^+ + 3ADP + 3P + \text{light} \rightarrow 2NADPH + 2H^+ + 3ATP + O_2$$

Light energy is used to synthesize ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate). Synthesis can take place in two forms: in cyclic or in non-cyclic pathways. Microalgae and cyanobacteria mainly use light energy in a non-cyclic reaction. In the non-cyclic pathway, light-harvesting antenna complexes (chlorophyll and other accessory pigments) of photosystem II capture photons.

Electrons are transported to a primary electron acceptor molecule. This happens when a chlorophyll molecule at the core of the photosystem II reaction center obtains sufficient excitation energy from the adjacent antenna pigments. The *Z-scheme* initially generates a chemiosmotic potential across the membranes. An ATP synthase enzyme uses the chemiosmotic potential to make ATP during photophosphorylation. On the other hand, NADPH is a product of the terminal redoxreaction in the *Z-scheme*. In Photosystem I, the electron enters a chlorophyll molecule and gets excited due to the light absorbed by the photosystem. A second electron carrier accepts the electron and passes it down, lowering energies of electron acceptors. Hydrogen ions move across the thylakoid membrane into the lumen through the influence of the energy created by the electron acceptors.

The cyclic reaction is similar to that of the non-cyclic, but differs in the form that it generates only ATP, with no reduced NADP (NADPH) being formed. The cyclic reaction takes place only in photosystem I. Once the electrons are displaced from the photosystem, the electrons are passed down the electron acceptor molecules and returns to photosystem I, from where they were originally emitted.

4.1.2. Light independent reaction

In the light-independent reactions or dark reactions, the enzyme RuBisCO captures CO_2 from the atmosphere. This process requires the newly formed NADPH, and is called the Calvin–Benson Cycle [104].

To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then used in a variety of ensuing processes forming other organic compounds. An example of this process is the formation of cellulose, which is the precursor for lipid and amino acid biosynthesis, or a fuel for respiration. The overall equation for the light-independent reactions is [105]

$$3CO_2 + 9ATP + 6NADPH + 6H^+ \rightarrow C_3H_6O_3\text{–phosphate} + 9ADP + 8P + 6NADP^+ + 3H_2O$$

The fixation or reduction of carbon dioxide takes place by combining carbon dioxide with a five-carbon sugar, ribulose 1,5-bisphosphate (Ru5BP). This yields two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). In the presence of ATP and NADPH (from light-dependent stages), GP is reduced to glyceraldehyde 3-phosphate (G3P).

This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Most (5 out of 6 molecules) of the G3P produced is used to regenerate Ru5BP so that the process can continue. The 1 out of 6 molecules of the triose phosphates is not “recycled” and often condenses to form hexose phosphates, which ultimately yields sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids.

4.2. CO_2 sources

Microorganisms formed via photosynthesis, such as microalgae, use CO_2 as a carbon source. No growth can occur without it. Insufficient supply of CO_2 is often the limiting factor of productivity. Based on the average chemical composition of microalgae biomass, approximately 1.8 t of CO_2 are required to produce 1 t of biomass. Microalgae can also produce proteins, fatty acids and dietary supplements for humans and animals. Furthermore, lipids from microalgae are chemically similar to those in common vegetable oils and are good potential sources for biodiesel. The microalgae-based biodiesel, in contrast to the one derived from fossil fuels, is renewable, biodegradable, and produced with low pollutant emissions [1]. Thus, reducing the atmospheric CO_2 by microalgal photosynthesis is considered safe and advantageous for the human ecosystem.

4.3. CO_2 fixation

Most of supply of the CO_2emissions can be traced to the flue gases produced by different industries. CO_2 can only be fixed on algae during the day time. Microalgae can eventually produce some CO_2 overnight as it happens with other plants. There is however and as a result a net positive CO_2 uptake.

In outdoor culture systems or open systems, an extra amount of flue gas supply is needed to provide the required amount of CO_2. Outdoor culture systems are limited by microalgal growth. These systems are not easy to control showing low productivity as a result of variability of the following: (a) environmental temperatures, (b) system circulation and (c) light utilization. In comparison with open culture systems, a closed photobioreactor is easy to control and can achieve high growth rates. A closed photobioreactor can be considered a bioscrubber for waste gas treatment. The microalgal cells cultured in this photobioreactor convert the CO_2 from the waste gas into biomass in an energy-efficient and economical manner [6].

The CO_2 fixation rate is related directly to light utilization efficiency and to the cell density of microalgae. Microalgal CO_2 fixation involves photoautotrophic growth in which anthropogenically derived CO_2 may be used as a carbon source. Therefore, biomass measurements or growth rate evaluations are critical in assessing the potential of a microalgal culture system for direct CO_2 removal [106,107].

The effects of CO_2 concentrations in air on microalgae growth have been evaluated in several studies in photobioreactors. The goal has been to consider CO_2 capture from waste gases at high CO_2 concentrations [78,108–113]. With this end, different air and CO_2 feed compositions were fed into the photobioreactor. This research has allowed the study of microalgal growth and CO_2 fixation, with this information being valuable to determine CO_2 removal efficiency. In spite of this, there is still lack of agreement on the optimum CO_2 concentration for various algal species.

The CO_2 removal efficiency in a photobioreactor with microalgal culture can be determined as the difference of CO_2 concentration of the incoming and outgoing effluents. The removal efficiency (%) can be thus determined using the following formula [5]:

$$\frac{\text{Influent of } CO_2 - \text{Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\%$$

The efficiency of CO_2 removal or fixation in a closed culture system depends on (a) microalgal species, (b) CO_2 concentration, (c) photobioreactor design and (d) operating conditions [78,106]. Cheng et al. [106] observed in a membrane photobioreactor, a maximum CO_2 removal efficiency of 55.3% at 0.15% CO_2 with a reduction of 80 mg/L h at 1% CO_2 in a *C. vulgaris* culture. In a three serial tubular photobioreactor, 27–38% and 7–13% of CO_2 was fixed by *Spirulina* sp. and *S. obliquus*, respectively, in cultures aerated with 6% CO_2.

On the other hand, in treatments with 12% CO_2 aeration, CO_2 fixation efficiency was only 7–17% for *Spirulina* sp. and 4–9% for *S. obliquus* [78]. In other words, there is a species dependence on the CO_2 efficiency removal or fixation. This may be due to physiological conditions of microalgae, such as potential for cell growth and CO_2 metabolism.

In studies by Yun et al. [102], the CO_2 fixation rate was determined from the carbon content of algal cells. The growth

rate was established as follows:

$$R_{CO_2} = C_C \times \mu_L \times \left(\frac{M_{CO_2}}{M_C}\right)$$

where R_{CO_2} and $\mu_L$are the fixation rate (g CO_2/m^3 h) and the volumetric growth rate (g dry weight/m^3 h), respectively, in the linear growth phase. $M_{CO_2}$and M_C represented the molecular weights of CO_2, and elemental carbon, respectively. The average carbon content (C_C measured by an elemental analyzer) (CHNS-932, Leco) was 0.507 g carbon/g dry cell weight. The algal growth rate was determined in the linear growth regime given that most of the algal growth occurred during this phase.

Due to climatic, land and water restrictions, it is challenging to collect and utilize microalgae directly on site. However, it is possible to increase the economics of microalgae utilization by using a two-stage process. In such a process, the CO_2 from a power generating stations or other source is first scrubbed (e.g. amine scrubber) and concentrated with a conventional process [114,115]. The resulting and concentrated CO_2 is then transported to a suitable site for microalgae production. This can be compared to the economics of other more "conventional" CO_2 capture process where CO_2 capture involves a separation process, followed by transportation and finally disposal in deep oceans, and/or depletion in gas wells.

One should mention that in this respect, some microalgae species are tolerant to relatively high temperatures (close and above 30 °C). This type of microalgae can be cultured in conjunction with the usage of high temperature flue gases from industrial neighboring sites [95]. These thermo tolerant strains may also simplify species-control. This is the result that that the optimum temperature of most microalgal species growth is in the 20–30 °C range. For instance, several unicellular green algal strains, identified as a species of Chlorella, were isolated from hot springs in Japan. These strains grew at temperatures of up to 42 °C and in air containing more than 40% CO_2. Their tolerance to both high temperatures and high CO_2 content makes them potentially appropriate microbial cells for photobioreactors involved in CO_2 capture from flue gases [95].

Table 4 reports the microalgae species identified as tolerant to moderate to high CO_2 concentrations and 20–30 °C temperatures. It is interesting to note that *Chlorococcum littorale*, a marine algae, showed exceptional tolerance to high CO_2 concentrations of up to 40% [116]. de Morais and Costa [78] reported that microalgae *S. obliquus*, *Chlorella kessleri* and *Spirulina* sp. also exhibited good tolerance to high CO_2 contents (up to 18% CO_2). This indicates their great potential for CO_2 fixation from CO_2-rich streams. For *Spirulina* sp., the maximum specific growth rate and maximum productivity were 0.44 d^{-1} and 0.22 g/L d respectively, with both using 6% and 12% CO_2 concentrations (v/v). The maximum cell concentration was 3.50 g/L (dry basis) at both CO_2concentrations. For *S. obliquus*, the corresponding maximum growth rate and maximum productivity were 0.22 and 0.14 g/L d. Murakami and Ikenouchi [116] developed an extensive screening, of more than 10 strains of microalgae with high capability of fixing CO_2. Two green algal strains, *Chlorella* sp. UK001 and *Chlorococcum littorale*, showed high CO_2 fixation rates exceeding 1 g CO_2/L d.

Table 4
Microalgae strains studied for CO_2 bio-sequestration (adapted from [95]).

Microalgae sp.	CO_2 (%)	Temperature (°C)	Biomass productivity (g/L d)	CO_2 fixation rate (L/d)
Chlorococcum littorale	40	30	N/A	1.0
Chlorella kessleri	18	30	0.087	0.163
Chlorella sp. UK001	15	35	N/A	> 1
Chlorella vulgaris	Air	25	0.040	0.075a
Chlorella vulgaris	15	–	N/A	0.624
Dunaliella	3	27	0.17	0.313
Haematococcus pluvialis	16–34	20	0.076	0.143
Scenedesmus obliquus	Air	–	0.009	0.0160
Scenedesmus obliquus	18	30 0.	14	0.26
Spirulina sp.	12	30	0.22	0.413

5. Wastewater treatment

5.1. Microalgal nutrition

Algae are important bioremediation agents. They are already being used by many wastewater facilities. The potential for algae in wastewater remediation is however much wider in scope than its current role. Microalgae contain higher nitrogen and phosphorus contents, approximately 10–11%, respectively, on a dry weight basis. These types of nitrogen and phosphorous levels are several times greater than that of plants. Microalgae have also been used extensively to remove heavy metals from wastewater even though these could not compete commercially with ion exchange resins [117]. The use of microalgae has also attracted attention because microalgae have the ability to remove both CO_2 and NO_x during their growth [118]. Microalgae can also produce, as stated in the previous sections, potentially valuable biomass, which can be used as an animal feed additive, slow-release fertilizer and biodiesel feedstock [119,120].

5.2. Nitrogen

Nitrogen in the form of nitrates and ammonia is the most commonly found nitrogen containing chemical species. Ammonium is among the most common chemical forms of nitrogen that can be readily absorbed by microalgae. In this respect, a cheap source of nitrogen can be a used as a wastewater stream or as secondary treated wastewater.

5.3. Phosphorous

Phosphorus is also an important element required for cell growth and microalgae metabolism. Phosphorus is an essential element included in DNA, RNA and ATP, and cell membrane materials. In the photophosphorylation process, phosphorus is an essential element contributing as ATP. As a result, phosphorus availability has a large impact in microalgae growth as it is considerably affected in photosynthesis. Lipid accumulation may occur in the culture media under phosphorus starvation conditions. Phosphorus is usually available in the wastewater as inorganic anions species such as $H_2PO_4^-$ and HPO_4^{2-} [121].

5.4. Wastewater components

Many species of microalgae are able to effectively grow in wastewater conditions due to the abundance of nutrient inorganic species. A major requirement for wastewater treatment (aside from sludge removal via conventional processes, such as activated sludge) is the need of removing high concentration of nutrients. This is true in particular for nitrogen (N) and phososphorus (P) species. Not doing so leads to eutrophication with nutrients accumulation in rivers, lakes or ponds. In the conventional

Table 5
Initial wastewater characteristics comparison between dairy/livestock wastewater and municipal wastewater [130].

Wastewater characteristics	Dairy wastewater		Municipal wastewater
	10% dilution	20% dilution	No dilution
TSS (mg/L)	283	135	93
VSS (mg/L)	220	120	58
pH	7.9	7.7	7.2
Ammonium as N (mg/L)	30.5	16.3	39
Nitrate as N (mg/L)	0.01	0.05	< 0.01
Nitrite as N (mg/L)	< 0.01	0.04	< 0.01
Organic nitrogen (mg/L)	50.7	20.2	12
TKN (mg/L)	81.0	36.5	51
Total nitrogen (mg/L)	81.0	36.6	51
Phosphate as P (mg/L)	2.6	1.8	2.1

Table 6
Average influent and effluent data ± SE (*n*) for seven farm digesters [133].

	Average influent	Average effluent	Percent decrease/increase
Temperature (°C)	26.2 ± 0.2 (74)	26.1 ± 0.1 (80)	0.4% decrease
Conductivity (mS/cm)	1.59 ± 0.1 (74)	1.73 ± 0.1 (80)	8.8% increase
pH	7.34 ± 0.1 (74)	6.64 ± 0.04 (80)	9.5% decrease
DO (mg/L)	0.21 ± 0.1 (73)	0.54 ± 0.1 (80)	157% increase
BOD (mg/L)	467 ± 40 (74)	96.2 ± 11 (80)	79.4% decrease
COD (mg/L)	2970 ± 260 (73)	472 ± 40 (79)	84.1% decrease
Turbidity (NTU)	1820 ± 200 (73)	172 ± 15 (80)	90.5% decrease
TSS (mg/L)	2210 ± 223 (72)	319 ± 56 (80)	85.6% decrease
NO_x–N (mg/L)	0.92 ± 0.2 (71)	0.18 ± 0.03 (80)	80.4% decrease
PO_4–P (mg/L)	13.3 ± 1.7 (74)	15.4 ± 1.4 (80)	15.8% increase
NH_4–N (mg/L)	46.1 ± 5.1 (72)	82.2 ± 5.0 (79)	78.3% increase
TKN (mg/L)	306 ± 28 (35)	166 ± 13 (37)	45.7% decrease

activated sludge process, phosphorus removal is particularly challenging [122].

Microalgae are efficient species in removing nitrogen, phosphorus and other toxic materials from wastewater. As a result, microalgae can play an important role during the final steps of wastewater treatment when nitrogen, phosphorous and chemical oxygen demand (COD) have to be reduced. Indeed, algae-based treatments have been found to be equally efficient at removing P species from wastewater, as compared to chemical processes [123].

The treatment of livestock effluents is also receiving increasing attention. Uncontrolled nutrients discharges have caused severe episodes of eutrophication in aquatic ecosystems and pollution of strategic groundwater resources [124]. Intensive farming together with the high carbon and nutrient concentration of livestock wastewaters (two orders of magnitude higher than domestic wastewaters) has surpassed the natural capacity of the surrounding environment to cope with these effluents [125]. Table 5 provides a comparison of municipal and livestock wastewater data [126].

The development of cost-effective and environmentally friendly methods for the treatment of livestock effluents is, thus, required. In this context, the algal–bacterial system application for the recovery of livestock effluents provides an in situ microalgal-O_2 production. This occurs via photosynthesis and through nutrients recycling with N and P assimilation into the algal–bacterial biomass [127]. Thus, when irradiated with natural light, microalgae produce the O_2 needed by aerobic bacteria to mineralize organic matter and oxidize NH_4^+. In turn, microalgae can consume the CO_2 released by bacteria, which mitigates CO_2 emissions [128,129].

5.5. Anaerobic digested dairy wastewater treatment

Treatment of livestock effluents is receiving increasing attention [131,132]. Biogas is derived through anaerobic digestion of biomass, such as animal wastes, municipal wastewater, and landfill waste. Anaerobic digestion is themicrobially mediated biochemical degradation of complex organic material into simple organics and dissolved nutrients. Digesters are physical structures that facilitate anaerobic digestion by providing an anaerobic environment for the organisms responsible for digestion. Processing livestock manure through anaerobic digesters captures methane, which can be used as an energy source while reducing emissions of this greenhouse gas [133].

Lansing et al. [133] studied 12 influent and effluent wastewater parameters to determine their statistically trends. This study was conducted -using seven digesters to assess variability within these systems. These digesters were identical in construction materials, but differed in length, wastewater management styles, wastewater sources, and hydrologic loading. During the digestion process, all of the organic matter and solid variables showed important reductions (Table 6). The average chemical oxygen demand (COD) of the influent wastewater decreased by 84.1% from 2970 mg/L to 472 mg/L, while the biological oxygen demand (BOD) decreased by 79.4% to 96.2 mg/L. The average turbidity decreased by 90.5% from 1820 NTU (Nephelometric Turbidity Unit) to 172 NTU, and the total suspended solids (TSS) concentration decreased by 85.6% L to 319 mg/L (Table 2). Dissolved nutrient (PO_4–P, NH_4–N) concentrations and conductivity increased as the wastewater moved through the digester (Table 6), with the NH_4–N concentration increasing by 78.3% to 82.2 mg/L. The dissolved oxygen (DO) concentration slightly increased in the digester, but both the average influent (0.21 mg/L) and effluent (0.54 mg/L) DO concentrations were anaerobic. The average pH decreased from 7.34 to 6.64.

It was shown that the digester effluent wastewater contains an important amount of colloidal solid particles/organic matters which are difficult to oxidize biologically [134,135]. These suspended colloidal particles also reduce the opacity of the cultured media. Thus, to slowly remove biodegradable COD, other treatment strategies should be applied. In this respect, coagulation is a very well-known process of removing colloidal particles present in wastewater, in order to achieve the aggregation of suspension particles by sedimentation or flotation. To facilitate the use of coagulant agents, aluminum or iron salt can be employed [16].

Dosta et al. [16] reported experiments for the reduction of COD (suspended solids) in aerobically digested effluent wastewater. Various $FeCl_3$ dosages (0–2500 mg/L) were added to the effluent. These authors performed coagulation/flocculation tests in a Jar-Test device (Flocculator 2000, KEMIRA Kemwater) involving a vigorous mixing period (30 s), a low mixing phase (15 min) and a final settling period (20 min). Both coagulant ($FeCl_3$) and flocculent agents were added to the desired concentration at the beginning of the first step (vigorous mixing period). The effectiveness of the coagulation/flocculation was evaluated by means of chemical oxygen demand (COD) and suspended solids (SS) reduction. Fig. 9 reports the average COD reduction yields obtained in those experiments. When $FeCl_3$ was dosed, suspended solids reduction yields were in the range of 8–10%. Due to the acidity of the $FeCl_3$, the pH change was also observed in Fig. 7.

Recovery of nutrients from the digester effluent wastewaters with the help of microalgal culturing has been investigated by a number of researchers. Wang et al. [135] studied the nutrient removal in a dairy farm (Haubenschild Farm) effluent wastewater. It was found that algal growths need a minimum level of light in the culture media to grow efficiently. Wang et al. [135] measured

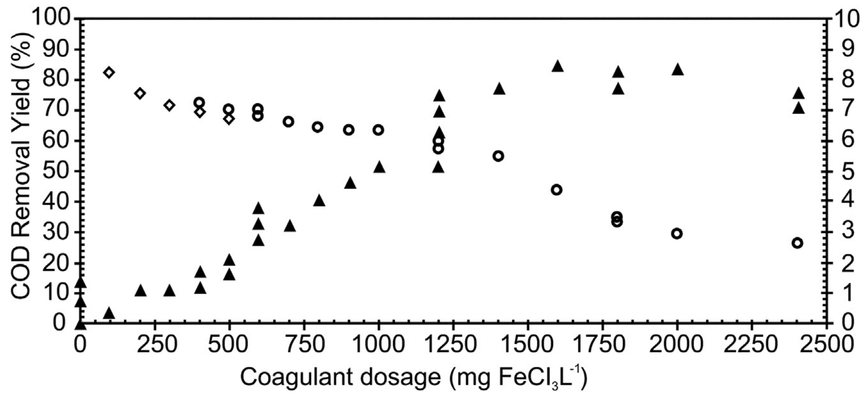


Fig. 7. Percentage of COD removal (▲) and effluent pH (O) during the jar-test using several $FeCl_3$ (Adapted from [16]).

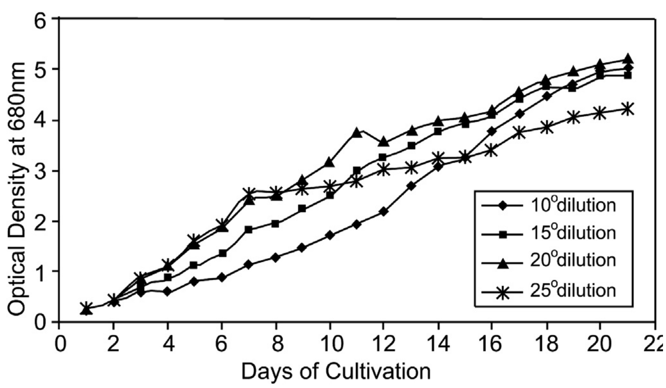


Fig. 8. Growth curve of *Chlorella* sp. in digested dairy manure with different dilution multiples (Adapted from [129]).

the optical density at 680 nm in 10 ×, 15 ×, 20 × and 25 × diluted digester effluents. Fig. 8 reported the wild isolated *Chlorella* sp. growth in all four diluted samples.

Comparing between them, they found that algae grew faster in the 20 × and 25 × diluted samples during the first 7 days. The 10 × sample increased the rate of growth after 12 days. Authors reported that the specific growth rates of the *Chlorella* sp. in those four specific samples were 0.282., 0.350, 0.407 and 0.409 g DW d^{-1}.

Most of the nitrogen in the dairy manure is found in the form of ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Results from the four dilution cases are reported by Wang et al. [129] as shown in Fig. 9. Total nitrogen was greatly reduced by 75–83% with, however, a fraction still remaining. This shows the presence of organic species left that could not be converted into ammonia and nitrogen associated species. A significant amount (62–75%) of total phosphorus was also found in all four samples. Concerning manure CODs, they are utilized to some extent by algae as carbon sources (27.4–38.4%) but not as efficiently as nitrogen and phosphorus are employed. These experiments were performed under axenic conditions and therefore, the reduction of COD could only be attributed its consumption by algae (Wang et al. [129]).

Treatment of livestock manure wastewater after being digested is necessary. This is due to the high waste water content of nitrogen, phosphorus and soluble total organic compound (TOC). From the above findings, it can be concluded that one can use microalgae in biological treatments of wastewater and that the final outcome is the production of a large amount of biomass.

5.6. Municipality wastewater treatment

Water used in our daily life goes down the drain and into the sewage domiciliary collection system. This waste water is normally designated as municipal wastewater. This includes water from baths, showers, sinks, dishwashers, washing machines, toilets and others. Small businesses and industries located in the urban areas also often contribute with large amounts of wastewater to sewage collection systems. Very frequently, large industries treat their own industrial wastewater. Therefore, industrial wastewaters remain contained in the industrial facilities and do not contribute to the municipal wastewater.

Most of the municipal wastewater treatment plants perform primary wastewater treatment systems consisting of (i) a screening chamber to get rid of large solids from the wastewater, (ii) grit chambers to remove the grit and (iii) settling tanks to allow the deposition of most of the particles at the bottom. Fig. 10 shows a typical wastewater treatment plant. Sludge from the settling tank is then sent to the digester plant for further treatment and for solid disposal. The remaining waste water is then further directed to the secondary treatment plant. In the secondary treatment plant, waste water is filtered further using a trickle filter. Following this step, wastewater is directed to the sedimentation tank or treatment ponds/lagoons. Relatively clear water from the sedimentation tanks is then sent to tertiary/advanced treatment units. Most of the tertiary treatment units are of the biological treatment type.

As mentioned, water from the secondary treatment processes is sent for a tertiary treatment to remove nutrients such as ammonia, nitrate and phosphate as well as some organic compounds. Tertiary biological wastewater treatment can be achieved using conventional aerobic treatment methods such as activated sludge. These processes are energy intensive given the need of O_2 supply.

Furthermore, they do not offer the opportunity for recycling the valuable nutrients present in wastewater [137]. On the other hand, although energetically more favorable than their aerobic counterparts, anaerobic processes are often limited by low ambient temperatures and the poor reduction of nitrogen and phosphorus [138].

Aerobic wastewater treatment processes are energy intensive given the need of mechanical aeration to provide oxygen to aerobic bacteria consuming organic matter. Aeration accounts for 45–75% of a wastewater treatment plant's total energy costs. In clear contrast with this, in algae based wastewater treatment, the cultivating algae provide the oxygen necessary for the aerobic bacteria. Thus, cultivation of algae can provide an efficient way to consume nutrients and provide the aerobic bacteria with the needed oxygen through photosynthesis. It is estimated that 1 kg of BOD removed in an activated sludge process requires 1 kWh of electricity for aeration with this producing 1 kg of fossil fuel derived CO_2 for power generation [139].

Wang [135] and his coworkers investigated the effects of green algae *Chlorella* sp. culture on the nutrient removal from four different

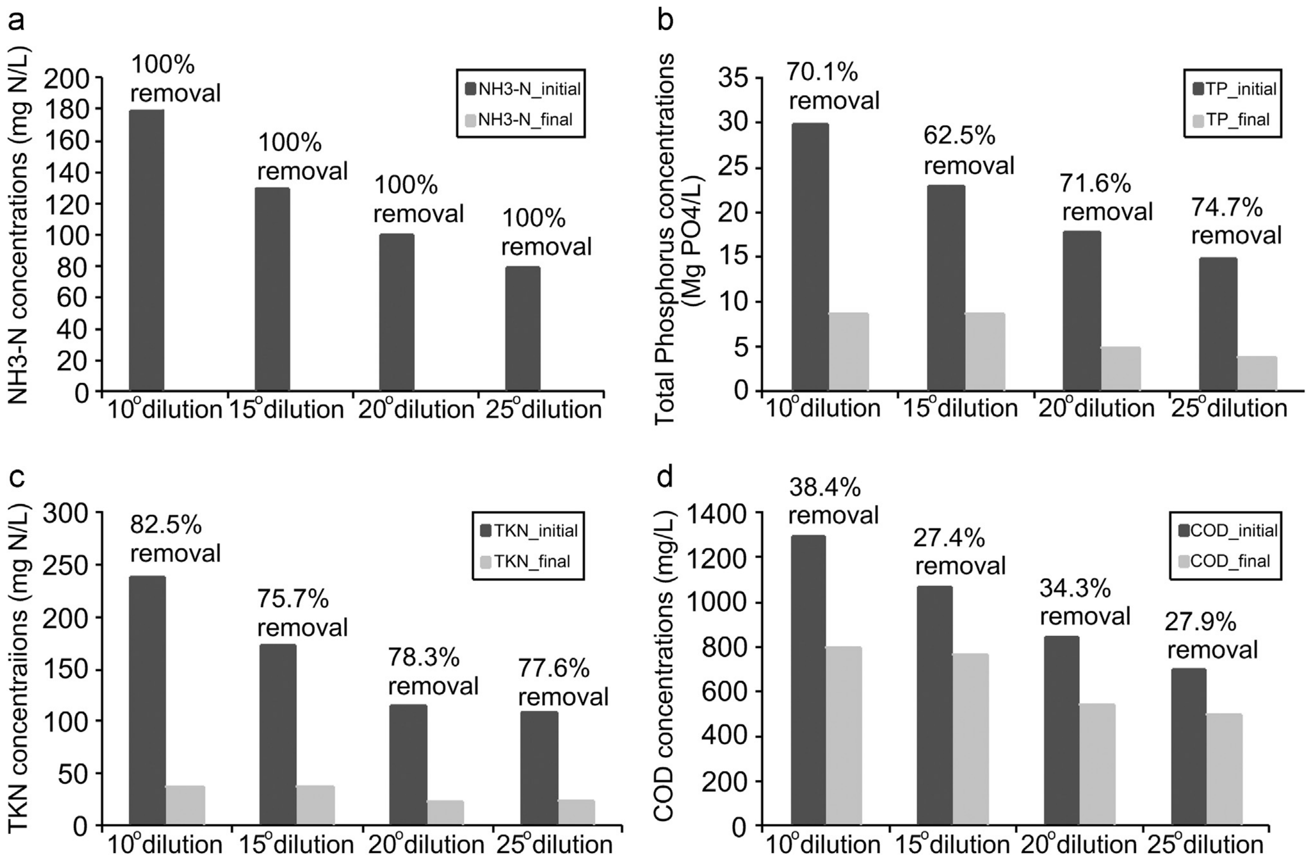


Fig. 9. (a) Initial and final ammonium concentrations and removal rates; (b) initial and final total Kjehldahl nitrogen (TKN) concentrations and removal rates; (c) initial and final total phosphorus (TP) concentrations and removal rates and (d) initial and final chemical oxygen demand (COD) concentrations and removal rates (Adapted from [129])

wastewater samples collected from St. Paul, MN, USA, Metropolitan wastewater treatment facilities. At first, they analyzed the water samples collected from different treatments units of the existing facilities. The analysis results are listed in Table 7. One can see from this table that the chemical characteristics of the wastewater leaving the primary settling unit remain similar to the one of the incoming wastewater.

However, the chemical composition of the water leaving both the aeration tank and the centrate from the sludge centrifuge is significantly different than those of the inlet streams of the respective units. One can note that the effluents are stabilized by the activated sludge process in which ammonium is oxidized to nitrate, phosphorus is absorbed, and COD is significantly reduced. The effluents in this case can be discharged with further disinfection such as chlorination being required. The centrate is generated using a physical separation process in the sludge centrifuge, with this liquid stream retaining high levels of ammonium, phosphorous, and COD.

Wang et al. [129] used green algae *Chlorella* sp. culture to remove nutrients from the waste water samples collected from different treatment process locations zones as reported in Table 7. It was reported that green algae *Chlorella* sp. successfully grew in all of the studied samples. From this study, it was concluded that green algae *Chlorella* sp. consumed ammonium or nitrate. These are two primary nitrogen nutrient sources already available in the wastewater samples.

Table 7 summarizes the nutrient removal from each wastewater sample after treatment with green algae *Chlorella* sp. From this table, it can be concluded that NH_4–N, the major nitrogen bearing inorganic compound species in the wastewaters before and after primary settling and centrate, was significantly reduced. In fact, removal for #1, #2, and #4 were 82.4%, 74.7%, and 78.3%, respectively. Reduction of phosphorus was up to 90% (Table 7) in wastewater #1, #2, and #4. This shows the effectiveness of algae growth as an effective removal system. However, only 4.7% phosphorus was removed from the effluent of #3.

Table 8 shows that up to 90% of phosphorus was removed from wastewater #1, #2 and #4 by algae growth. If one compares the inorganic N/P ratios for the four wastewaters treated, before and after algal cultivation (Tables 7 and 8), one can see that there is optimal inorganic 6.8–10 N/P ratio for algae growth, with this ratio dropping from 52.3 to 20.8. At the end of the experiment, one can expect severe phosphorus content limitations for algal culture. However, the unbalanced N/P ratio of the centrate did not affect nitrogen or phosphorous removal. This suggests that the N/P ratios and the absolute levels of N and P for both algal growth and effluents from wastewater should be considered when evaluating the nutrient composition effects on algal growth.

Arbib et al. [140,141] studied the algal growth rate and nutrient removal along with carbon dioxide biofixation using *S. obliquus* and *Chlorella stigmatophor*. These species were cultivated in urban wastewater at different nitrogen and phosphorus ratios, ranging from 1:1 to 35:1. These authors found that the nitrogen to phosphorus ratios ranging between 9 and 13 (263 and 322 mg/L d respectively) are very important to achieve optimum batch biomass productivity. Renuka et al. [142] worked with different microalgae groups. Their findings showed highest dry cell weight (0.97 mg/L) using *Calthrix* sp. with 57–58% NO_3–N, 44–91%PO_4–P removal from sewage wastewater.

Woertz et al. [143] conducted algal growth experiments using wastewater samples collected from the effluent of a secondary treatment facility at the San Luis Obispo, California, municipal wastewater treatment plant. Before adding the wastewater into the algae culture system, it was first circulated through a filter screen with 196-μm openings. This allowed removing micron size

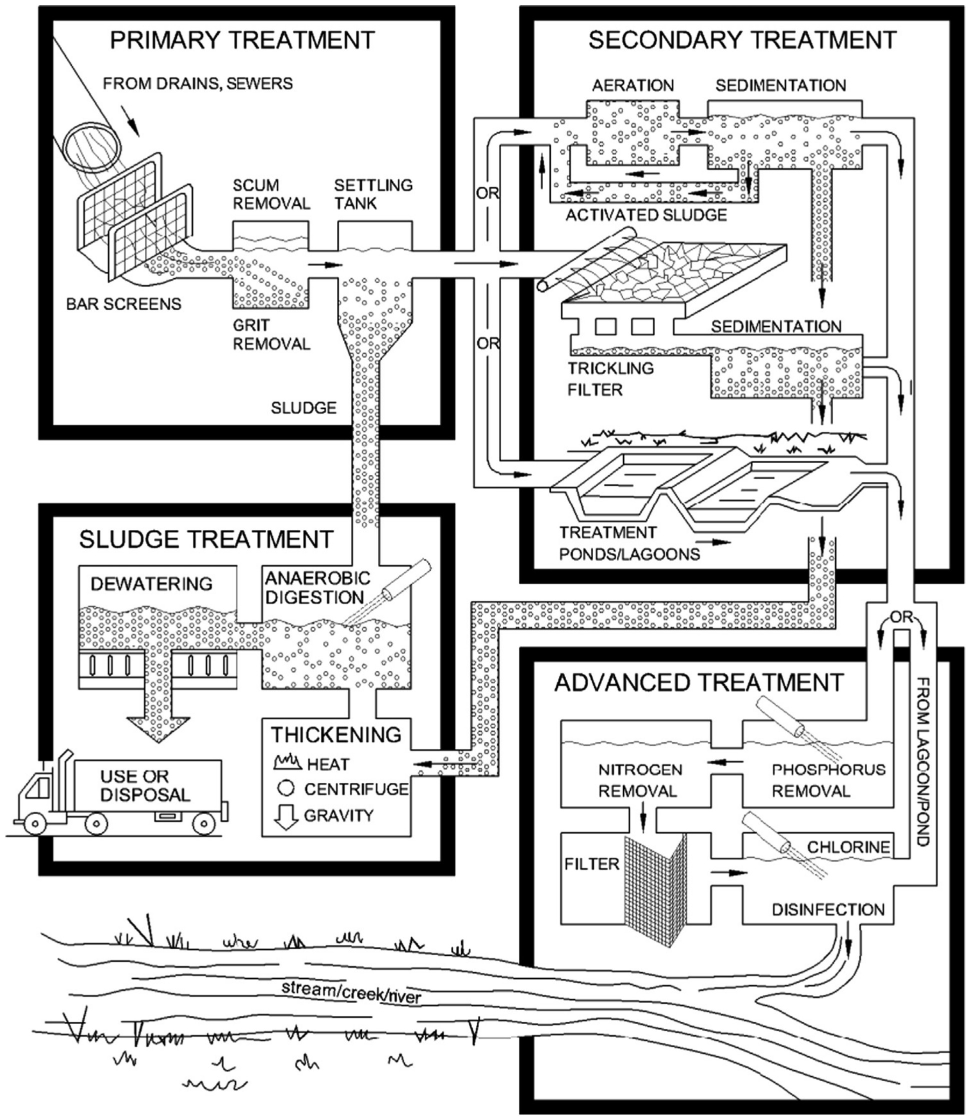


Fig. 10. Typical wastewater treatment process (Adapted from [136])

Table 7
Characteristics of four wastewater samples from the St. Paul Metropolitan Wastewater Treatment Plant [135].

Parameters	Wastewater before primary settling	Wastewater after primary settling	Effluent from aeration tank	Centrate from sludge centrifuge
NH_3–N (mg/L)	33.4 ± 0.6	32.2 ± 0.4	ND	71.8 ± 1.1
NO_3–N (mg/L)	ND (not detected)	ND	16.95 ± 0.07	ND
NO_2–N (mg/L)	ND	ND	0.074 ± 0.003	ND
TP (mg/L)	5.66 ± 0.08	6.86 ± 0.05	0.32 ± 0.04	201.5 ± 10.6
TN (mg/L)	40.65 ± 0.07	38.95 ± 1.91	19.1 ± 0.1	131.5 ± 2.1
COD (mg/L)	231.0 ± 4.2	224.0 ± 4.2	42.2 ± 1.9	2250.0 ± 99.0
Inorganic N/P	5.9	4.7	53.2	0.36

particles. Collected wastewater was then allowed to flow over a wedge-wire inclined screen to remove other fine solids. Finally, the water was treated in an anaerobic digester before being used in the algae culture experiments.

For the municipal wastewater, the culture volume in each Roux bottle was 800 mL. Wastewater was introduced into the bottles with a daily draw-fill procedure at the end of the light period. Three daily hydraulic loading rates were tested as follows: 200, 267, and 400 mL of primary effluent to achieve 4-, 3-, and 2-day hydraulic residence time (HRTs) , respectively. For the air–CO_2 sparge treatments, each HRT was run with duplicates. For the air-only treatment, the 3-day HRT was run over 99% ammonium. Orthophosphate removal was achieved in the CO_2-sparge treatment with both 3- and 4-day HRTs as reported by Woertz et al. [143](Table 9). To determine the fate of the removed ammonium and to validate the results, a nitrogen balance was done on four occasions over 10 days of operation.

A recent study characterized a *Chlorella species* called *Chlorella minutissima* which was identified in wastewater treatment oxidation ponds in India [144]. *C. minutissima* was able to grow well in high concentrations of raw sewage and dominated the subsequent pond stages in the oxidation pond system. Analysis has found that

Table 8
Nutrient removal rates by algal growth in four wastewater samples from the St. Paul Metropolitan Wastewater Treatment Plant [135].

Parameters	Wastewater before primary settling (%)	Wastewater after primary settling (%)	Effluent from aeration tank (%)	Centrate from sludge centrifuge (%)
NH_3–N	82.4	74.7	–	78.3
NO_3–N	–	–	62.5	–
NO_2–N	–	–	−6.297	–
PO_4–P	83.2	90.6	4.69	85.6
TN	68.4	68.5	50.8	82.8
COD	50.9	56.5	−22.7	83.0
Inorganic N/P	6.2	12.77	20.8	0.538

Table 9
Nutrient removal of municipality wastewater cultures [143].

	Total ammonia nitrogen (mg/L)			Phosphate as P (mg/L)		
	Influent	Effluent	% Removal	Influent	Effluent	% Removal
CO_2 4-day HRT	39.0	< 0.02	> 99	2.1	< 0.02	> 99
CO_2 3-day HRT	39.0	< 0.02	> 99	2.1	< 0.02	> 99
Air 3-day HRT	39.0	6.1 (± 0.89)	84	2.1	< 0.02	> 99
CO_2 2-day HRT	39.0	0.6 (± 0.57)	98	2.1	0.15 (± 0.15)	93

this species can grow heterotrophically in the dark, and mixotrophically in the light utilizing a variety of organic carbon substrates, over a wide pH range. Furthermore, *C. minutissima* can utilize either ammonia or nitrate as an N source. The growth of these algae was shown to be highest under mixotrophic (photoheterotrophic) conditions with biomass productivity of 379 mg/L after 10 days of growth compared to biomass of 73.03 mg/L under photoautotrophic conditions [135]. *C. minutissima* could, therefore, be a good candidate for high biomass productivity in a wastewater high-rate pond system. All of these experiments further demonstrate that chlorophytic microalgae such as *Chlorella* can grow well even in very raw wastewater environments.

Based on the above reviews and on the research done so far regarding the biological municipal wastewater treatments, it can be concluded that mass culture of microalgae in the presence of N and P in the municipality wastewater can convert N and P into algal biomass.

5.7. Industrial wastewater treatment

The composition of the industrial wastewaters differs from one disposal site to another and considerably from domestic waste composition. Table 10 shows the typical range of the biochemical oxygen demand (BOD) and the total suspended solids (TSS) load in industrial wastewaters.

Industrial wastewaters contain heavy metals such as cadmium, chromium, zinc and others. Furthermore, they contain organic chemical toxins such as hydrocarbons, biocides and surfactants. Effluents from textile, leather, tannery, electroplating and other metal processing industries have considerable amounts of toxic metal ions. These types of toxic elements are both harmful to the ecosystem and unsafe for humans. The conventional methods of industrial wastewater treatments involve precipitation, ion exchange, electrowinning (electroextraction) and electrochemical methods [146].

Table 10
Typical range of BOD and TSS load in industrial wastewaters.
Source: Industrial wastewater treatment plants self-monitoring manual, Chapter 2, 2002 [145].

Origin of waste	Biochemical oxygen demand (BOD) (kg/ton)	Total suspended solids (TSS) (kg/ton product)
Dairy industry	5.3	2.2
Yeast industry	125	18.7
Starch and glucose industry	13.4	9.7
Fruits and vegetable industry	12.5	4.3
Textile industry	30–314	55–196
Pulp and paper industry	4–130	11.5–26
Beverage industry	2.5–220	1.3–257
Tennary industry	48–86	85–155

Due to generally low nitrogen and phosphorous concentrations and high toxin levels, algal growth rates are lower in many industrial wastewaters while compared with their growth in municipal domiciliary wastewaters. Consequently, there is less potential for utilizing industrial wastewaters for algal culture. Industrial wastewaters contain chemicals and pigments. These wastewaters also contain metals, P and N, at low concentrations and are able to support algal growth with *B. braunii* and *Chlorella saccharophila*, and a marine alga *Pleurochrysis carterae* [147].

6. Algae growth/cultivation

Microalgae culture offers important advantages to improve utilization efficiency of solar energy and CO_2 fixation. Since the culture can be operated in continuous mode, it allows maximum annual productivity. The cells structures of microalgae are relatively small and the growth rate of microalgae is much faster than other crops. Thus, the harvesting time of microalgae is pretty short which allows faster CO_2 capture. For an equivalent amount of microalgae production, much smaller areas of land are required as compared to other crops. Therefore, microalgae are unique because they combine the capturing ability of photosynthesis with the high yield of controlled microbial cultivation [148].

The optimization of strain-specific growth/cultivation is a complex topic with many interrelated factors affecting it. These include among others, the following reaction engineering parameters: (a) light (day–night cycle and irradiation intensity), (b) temperature, (c) nutrient concentration, (d) O_2, (e) CO_2, (f) pH, (g) salinity, (h) water quality, (i) mineral and carbon regulation/bioavailability, (j) cell fragility, (k) cell density and (l) growth inhibition.

Furthermore, there are other culture growth, reactor design and operation issues that affect growth such as (a) mixing, (b) fluid dynamics and hydrodynamic stress,(c) culture depth, (d) gas bubble size and distribution, (e) gas exchange, (f) mass transfer, (g) dilution rate, (h) toxic chemicals and pathogens (bacteria, fungi, viruses) and (i) competition by other algae and harvest frequency. Thus, for large scale cultivation, algae should have desirable features. These are summarized in Table 11 [149].

High cell density cultures can be achieved using microalgae biotechnology with proper reactor design and process optimization. For this reason, it is important to develop the general knowledge in this field, with emphasis on the most critical scale-up and operational parameters such as (a) light irradiation, (b) mass transfer, (c) shear forces and (d) mixing rate. These

Table11
Desired characteristics of algae for mass culturing [149].

Algae characteristic	Advantages	Disadvantages
(1) Growth in extreme environments	Reduced problems with competing species and predators	Only limited numbers of species available. Culture difficult to maintain on a large scale under extreme environments (i.e. cold weather)
(2) Rapid growth rate	Provides competitive advantage over competing species and predators. It has a reduced pond area required	Growth rate is usually inversely related to cell size; i.e. fast growing cells are usually very small in size
(3) Large cell size, colonial or filamentous morphology	Reduces harvesting costs	Large cells usually grow slower than smaller size cells
(4) Wide tolerance of environmental conditions	Less control of culture conditions required for reliable culture	
(5) Tolerance of shear force	Allows cheaper pumping and mixing methods to be used	
(6) High cell product content	Higher value of biomass	Products are usually secondary metabolites (not directly involved in normal growth). High concentrations of secondary metabolites normally mean slower growth

Table 12
Light penetration depth[a] (cm) into cultures of *Nannochloropsis* sp. as effected by the concentration of cell mass [150].

DW (g/L)	2	10	50
Blue (410–450 nm)	0.96	0.19	0.04
Green (580–600 nm)	9.43	1.89	0.38
Red (670–678 nm)	1.25	0.25	0.05

[a] To the depth in which light energy is 10% incident light

parameters are closely interrelated and they determine the productivity and efficiency of a specific reactor unit.

Moreover, the maximization of the algal productivity also implies optimizing algae culture; maximizing CO_2 capture and wastewater treatment. One of the major issues in the industrial scale-up of microalgae culture is related to algae culture in open ponds under uncontrolled conditions. It is only recently that closed bioreactors have been considered complementary to algal mass culture in open systems. The following are the key aspects of the closed system reactor design: (i) light source and orientation; (ii) algae circulation (iii) materials of construction, (iv) CO_2 feed and O_2 removal and (v) pH and temperature control.

6.1. Effects of solar irradiation

Efficient utilization of solar irradiation by cells is one of the major objectives of an economically viable microalgae culture system. A cost-effective system whether open or closed, is characterized by a high surface area as well as by volumetric productivity. This can be achieved by establishing an optimal solar irradiation regime in the culture.

Table 12 summarizes the light penetration rate in the culture of *Nannochloropsis* sp. at different wavelength ranges of incident light [150]. From this table, one can see that the light penetration into the culture decreases exponentially as the cell density increases. Generally, the light penetration is expressed as a percentage of total incident irradiation impinging on the culture surface. Two light zones are thereby affected in the photobioreactor: (a) the illuminated volume in which light supports photosynthesis, and (b) the dark volume, in which light intensity is below the compensation point and net photosynthesis cannot take place. One can note that the higher the algae population density, the smaller the light path and the more complex it becomes to attain efficient utilization of solar irradiation, (i.e. an even distribution of light to all cells in the reactor).

In this respect, one should mention that algae have developed several mechanisms like plants, to adjust to changes and quality of the light and intensity. However, the adjustment capacities vary from species to species. Algae with phycobilisomes may prefer low light intensities (i.e., ~10 mmol photons $m^{-2}\,s^{-1}$). Some other algal strains (e.g., most dinoflagellates) often need higher light intensities (~60–100 mmol photons $m^{-2}\,s^{-1}$). Colorless algae such as *astasia*, *polytomella* and *prototheca* are best kept in a closed cupboard. These algae have otherwise the same maintenance requirements as their photoautotrophic relatives. To develop a culture of organisms from extreme environments, specialized literature should be consulted [151]. Standard light intensities between 10–30 mmol photons $m^{-2}\,s^{-1}$ have proven to be appropriate in combination with temperatures commonly used for long-term culturing of most microalgal species. One should note, however, that over-illumination is a widespread misunderstanding for sustainable maintenance of cultures. Not only can excessive irradiation result in photo-oxidative stresses in some algae, but localized heating may also be a problem. Moreover, light and dark photoperiods are required for the maintenance of most cultures. Some algae (e.g. many tropical open-ocean *coccolithophorids*) may be destroyed by continuous irradiation [152]. In most algal cultures, light/dark periods are required to vary from 12/12 to 16/8 hours of light to hours of darkness [153]. Inappropriate hours of light to hours of darkness may lead to unwanted photoperiodic effects. For example, short day length periods may cause cyst formation in marine dinoflagellates like *Lingulodinium polyedrum*. These cysts are germinate with difficulty under standard culture conditions [154].

Regarding the way that light affects mass cultures of phototrophic microorganisms, growth response of mass cultures has been erroneously described using the so-called 'light curve' [155]. This provides a generalized shape of the light response curve, relating the photosynthetic or growth rate of the culture to the intensity of the light source (i.e. the photon flux density (PFD) impinging on the culture surface). The light source is thus considered as the sole rate-limiting factor in an irradiation limited system. This relationship, however, is only correct for optically thin cultures, of low density population where the cell mutual shading is essentially absent. In reality, however, mass cultures exposed to high irradiation density cannot be maintained in optically thin concentrations with no mutual shading. This is true since PFD is much higher outdoors, up to an order of magnitude or more, than the photosynthetic saturating light intensity. Excess light may cause photo inhibition followed by culture death. The most practical approach by which to cope with this phenomenon is to increase cell density to the point at which mutual shading causes cells to receive strong light intermittently.

The high PFD prevailing outdoors is thereby 'diminished' or 'diluted' for the individual cells. Light energy reaching each individual cell during a long exposure time is thus not only a function of the light source intensity, but is also and often more so, dependent on cell density. Thus, changes in growth rate may indeed be manifested in a culture as a response to a given intensity of light [155]. A major parameter in mass culture is the output rate of cell mass. This output rate of cell mass in continuous cultures at steady-state is a function of both the growth rate and cell density. In this respect, inadequate selection of optical path and culture density may yield output rates significantly below maximal values.

As in any biological phenomenon related to optimal exploitation of resources per unit irradiation area, there is a certain optimal algal content which may lead to a highest output rate. Definition of this parameter involves an 'optimal cell density' (OCD, g/L), this optimal cell density is system specific and is required to be maintained in a culture media to exploit sun irradiation most efficiently [150].

The growth rate, k, can be calculated from the integrated growth equation proposed by Sorokin and Kraus [156]:

$$k = \log_2 \frac{\text{O.D.}_1}{\text{O.D.}_0} \times \frac{1}{t}$$

where O.D._0 and O.D._1 are optical densities at the beginning and end of the time interval, t.

By using a base 2 logarithm and the day unit of time, the growth constant, k, becomes equivalent to the number of doublings per day. At least five readings need to be taken during exponential growth in each experiment at a particular light intensity. Reproducibility of the rate in at least two subsequent experiments is required. It is recommended to calculate the logarithmic growth rate for each light intensity as an average from two experiments, with each experiment conducted induplicate.

Fig. 11 shows the effect of light intensities on the growth of different algae species as reported by Sorokin and Kraus [156]. The growth effect can be categorized in three distinct regimes: (i) a light dependent regime in which growth rate increases, (ii) a light independent regime where the growth rate stabilizes, (iii) a light independent regime where growth rate declines. In this respect, a high algae growth rate was found when using low light intensities. Thus, microalgae are suitable for culturing in shaded areas.

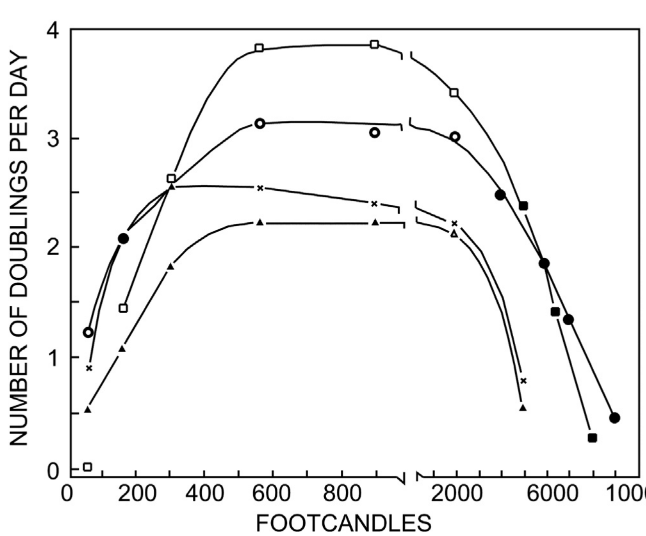


Fig. 11. The growth rates of four species of algae at 25° C measured at limiting, saturating, and inhibiting light intensities. The symbols are as follows: *Chlorella pyrenoidosa* (van Niel), circles; *Chlorella vulgaris*, crosses; *Scenedesmus obliquus*, triangles; and Chlamydomonas reinhardti, squares. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light (Adapted from [156]).

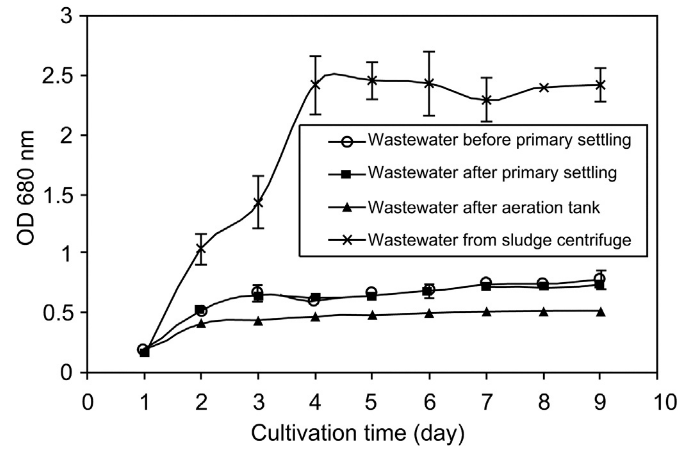


Fig. 12. Algae growth curves in the four wastewaters (Adapted from [135])

Irradiation efficiency must also be considered when predicting mass culture yields. This is due to algal population density often limiting light intensities penetrating into the algal culture. Furthermore, the transition from a light dependent growth regime to alight independent growth phase may be significantly affected when changing the type of microalgae.

The light-independent plateau for the van Niel strain of *C. pyrenoidosa* and *Scenedesmus* extended from 500 to about 2000 ft-cd. *Chlamydomonas* however, stretched out in shorter strands from 500 to 1000 ft-cd. A short plateau extending from 250 or 300 ft-c to not more than 600 ft-c was also observed, with this plateau being characteristic of *C. vulgaris* [156]. Low light-saturating intensity and negative low intensity effects to strain growth are characteristic features also observed in shade plants [156].

In Fig. 12, the growth curves of four different municipal wastewater samples are reported as shown by Wang et al. [135]. Table 8 shows the different characteristics of the samples collected from the municipal wastewater treatment plant at St Paul, Minnesota, USA. The optical density of the four samples was established at 680 nm. Wang et al. [135] cultured *Chlorella* sp. in those four specific samples under axenic conditions. It was noted that *Chlorella* sp., survived in all four cases. Similar growth patterns in the first 3 days were followed by an exponential phase lasting 1 extra day. This behavior took place before entering the secondary growth phase. One can note that algae in the four wastewater samples before and after primary settling displayed growth curves close to each other. The growth curve of the wastewater from sludge centrifuge was found to be higher than all other three culture systems. This behavior was assigned to the similar chemical composition type, as reported in Table 8. Moreover, algal growth was significantly enhanced in the centrate media due to the much higher nitrogen and phosphorous available and due to the higher COD level. The average specific growth rates in the first 3 days were 0.412, 0.429, 0.343, and 0.948 d^{-1} in wastewaters before and after primary settling, in effluent and in centrate, respectively.

Xue et al. [157] used vertically installed optical fibers to monitor the culture flow direction. A light source was placed inside the set up to promote a "flashing light effect" (FLE) on microalgae, so as to obtain high light efficiency. Three types of optical-fiber photobioreactors involving FLE of microalgae were studied, i.e. air-driven panel, pump-driven panel and stirred tank type reactors. Results demonstrate that with light/dark 10 Hz frequency cycles, the microalgae productivity was increased by 43% and 38% for *Spirulina platensis* and *Scenedesmus dimorphus* respectively.

6.2. Effects of CO_2 concentration

The effect of CO_2 concentration on the growth of *C. vulgaris* was studied by Yun et al. [4]. The inoculums were prepared by bubbling air. The growth was somewhat inhibited at 15% (v/v) CO_2 with air. However, when the inoculums were adapted to 5% (v/v) CO_2 with air, best growth was achieved [4],

Since the typical concentration of CO_2 in flue gas is around 15% (v/v), adaptation of *C. vulgaris* to higher CO_2 concentrations is needed for the direct use of flue gas. Moreover, it was found in a separate experiment that the gradual increase of CO_2 concentration gave even better growth at CO_2 concentrations of up to 30% 2 (v/v).

6.3. Effects of temperature

After light, temperature is the most important factor for microalgae culturing for both open and closed culture systems [158–160]. Like other microorganisms, microalgae have an optimum growth temperature, where maximum growth rate can be achieved. The growth rate dependency on temperature varies from species to species [161]. Some species can grow successfully at higher temperatures while others can tolerate very low temperatures. Microalgae can tolerate a range of temperatures and their response to temperature variations can affect the following (i) nutritional requirements, (ii) rates and nature of metabolism and (iii) cell compositions [162]. Usually, most of the microalgae species can tolerate temperatures up to 15 °C lower than their optimal temperature. However, exceeding the optimum temperatures by only 2–4 °C may result in the total culture loss [162].

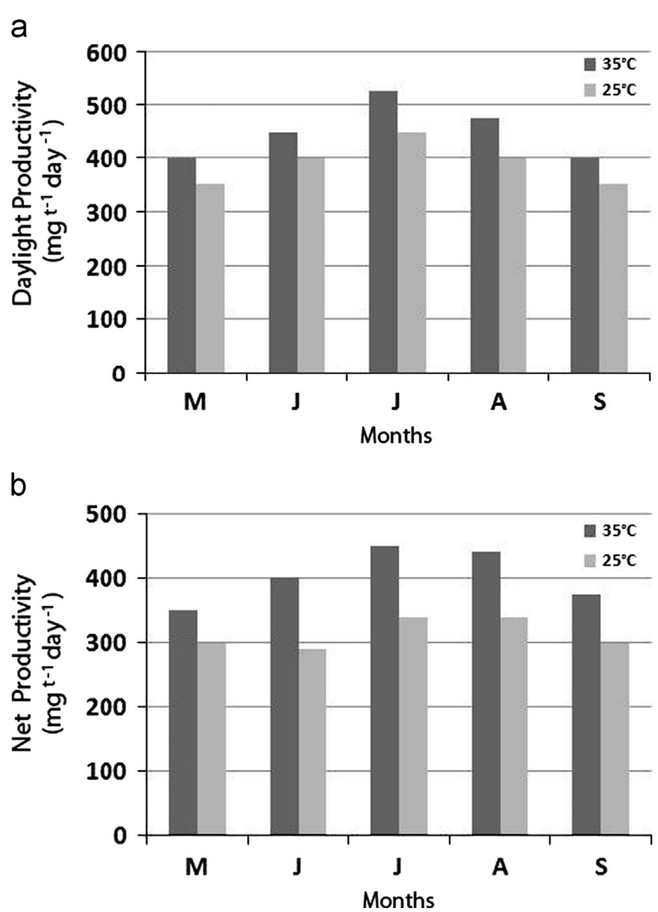


Fig. 13. Influence of temperature on the productivity of *Spirulina platensis* during (a) the daylight period and (b) 24 h day–night cycles (Adapted from [159]).

Table 13
Percentage of controlled growth of *Chlorella vulgaris* after 96 h exposure to pH modified Bristol's Medium [163].

Initial pH	% Growth	Final pH
3.0	27.3 ± 0.16	2.8
4.0	36.3 ± 0.17	4.0
5.0	55.6 ± 0.18	4.9
6.2	91.9 ± 0.10	6.2
6.9	100	6.9
7.5	124.9 ± 0.20	6.9
8.0	120.0 ± 0.18	7.8
8.3	46.1 ± 0.18	7.9
8.5	49.7 ± 0.18	8.1
9.0	37.2.0 ± 0.17	8.5

Torzillo et al. [159], reported that laboratory experiments have shown that the maximum biomass yield occurred when the temperature of *spiriluna* (algal species) was 35 °C. These authors carried out experiments outdoors from May to September and analyzed temperature effect on growth rate. During this period, the average biomass productivity was found to be 14% superior at 35 °C versus the growth rate observed at 25 °C (refer to Fig. 15). It is also shown in Fig. 13 that in the culture grown at 25 °C average biomass productivity decreases and biomass loss during the night was significantly higher (7.6% dry weight) than in the culture grown at 35 °C. This biomass loss accounts for the marked difference in the net biomass productivity of the two cultures (23% average).

6.4. Effects of pH and media composition

The pH is an important factor in culture media. In fact, common problems associated with culture media are the use of an inadequate pH and high levels of precipitate resulting from incorrectly formulated media, including omission of vital ingredients (e.g., silicon for diatoms, vitamins) [163]. Most algae are tolerant of fairly large changes in pH. However, if the inoculums vigor is suboptimal, then poor or no growth can result. In most cases, freshwater eukaryotic algae prefer acidic environments (pH 5–7), whereas cyanobacteria prefer alkaline environments (pH 7–9). Furthermore, high levels of precipitate can result in nutrient limitation and osmotically stressful microenvironments [163].

Rachlin and Grosso [163], measured the percentage of controlled growth of *C. vulgaris* incubated for 96-h at various pHs, as shown in Table 13. It can be seen that for the acidic and neutral pHs, the pH remained relatively constant over the 96-h incubation period. However, for alkaline pHs (pH 7.5 and greater) cell growth appeared to change the pH reducing it to 7.0–7.9, with this change being a function of the initial pH. In this respect, one should notice that at alkaline pHs microalgae cell growth during 96-h incubation periods (Table 13) is higher than the acidic media.

Table 13 shows that the pH control at 6.9 gave the 100% reference growth.Table 13 also shows that under acidic conditions (pH 3.0–5.0), *C. vulgaris* growth is reduced to 27.3–55%with respect to the growth at 6.9 pH. As well at the alkaline pH of 8.3–9.0, growth is reduced from 46% to 37.2% of the growth reference values of particular interest is that at the alkaline pHs of 7.5 and 8.0, the growth exceeds reference growth, indicating an optimum growth conditions within this narrow pH range.

7. Growth kinetics

The algae growth rate is a function of (a) the light intensity (I), (b) the temperature, (c) the nutrients and (d) the pH [155]. Thus, a specific growth rate of algae in outdoor cultures can be defined by

Goldman [155] as

$$\mu = \frac{ln2}{g} = \frac{1}{X}\frac{dX}{dt}$$

in which μ is the specific growth rate [T^{-1}], $ln\,2$ is the natural logarithm of 2, g is the cell generation period [T], X is the biomass concentration [$M\,L^{-3}$], and dX/dt is the change in biomass concentration with respect to time, or the biomass productivity per unit volume [$ML^{-3}\,T^{-1}$].

Algal productivity can be normalized on a per unit area basis by considering the culture volume V [L^3] with the culture area A [L^2] and depth d [L]. The total production of biomass B (XV) [M] per unit time is dB/dt [MT^{-1}]. This later term can be divided by the area A [L^2] to give P, the per unit areal yield [$ML^{-2}\,T^{-1}$]

$$P = \frac{1}{A}\frac{dX}{dt}V = \frac{1}{A}\frac{dB}{dt} = \mu X d$$

Nutrient effect on algal growth rates using the Monod model has been successfully tested by some research groups, as reported by Goldman and Carpenter [161]. The main basic assumption using the Monod model is that the growth rate of algae is solely dependent on the concentration of the limiting nutrient. Based on this assumption, the Monod model as defined by Goldman and Carpenter [161] is as follows:

$$\mu = f(S) = \hat{\mu}\left[\frac{S}{K_S + S}\right]$$

where S is a the limiting nutrient concentration and $K_S$is the half saturation coefficient, mg/L.

The Monod model does not explicitly include other effects such as light intensity or temperature. Goldman and Carpenter [161] considered, in this respect, that the $\hat{\mu}$ maximum growth rate in the Monod equation is not affected by nutrient concentration.

The $\hat{\mu}$ maximum growth rate is a function of other environmental variables such as light and temperature. When light intensity is held constant then the growth rate becomes solely a temperature function following an Arrhenius equation:

$$\hat{\mu} = Ae^{-E/RT}$$

where A is a constant in d^{-1}; E is an activation energy in cal mol^{-1}; R is the universal gas constant in cal K $mole^{-1}$and T is the temperature in Kelvin.

By substituting this equation into above equation, as proposed by Goldman and Carpenter [161], one can conclude the following:

$$\mu = Ae^{-E/RT}\left[\frac{S}{K_{S(T)} + S}\right]$$

Fig. 14a shows the increase of S nutrient concentration with temperature and light intensity held constant. This yields an algal growth rate augmenting first and reaching a plateau later. In this respect, Goldman [155] showed that growth is a first order function $\mu = \gamma_S S$ at low nutrient levels becoming of zero order $\mu \approx \hat{\mu}$ at higher nutrient concentrations. The intersection of the linear approximation for the first order portion of the growth curve with the horizontal asymptote gives $\hat{\mu}$and K_S. K_S represents the half-saturation coefficient, or the nutrient concentration$=0.5\hat{\mu}$.

Fig. 14b describes the effect of increasing light intensity while keeping nutrient concentration and temperature constant. The growth rate also displays a first order $\mu = \gamma_I I$ at low light intensity becoming of zero order $\mu \approx \hat{\mu}$ at high irradiation. Regarding the I_K parameter, one can observe that it represents K_S. Based on the Goldman and Carpenter [161] an Arrhenius type empirical equation is suitable for representing temperature effects:

$$\hat{\mu} = (1.8 \times 10^{10})e^{-6842/T}$$

where T is the absolute temperature in Kelvin units.

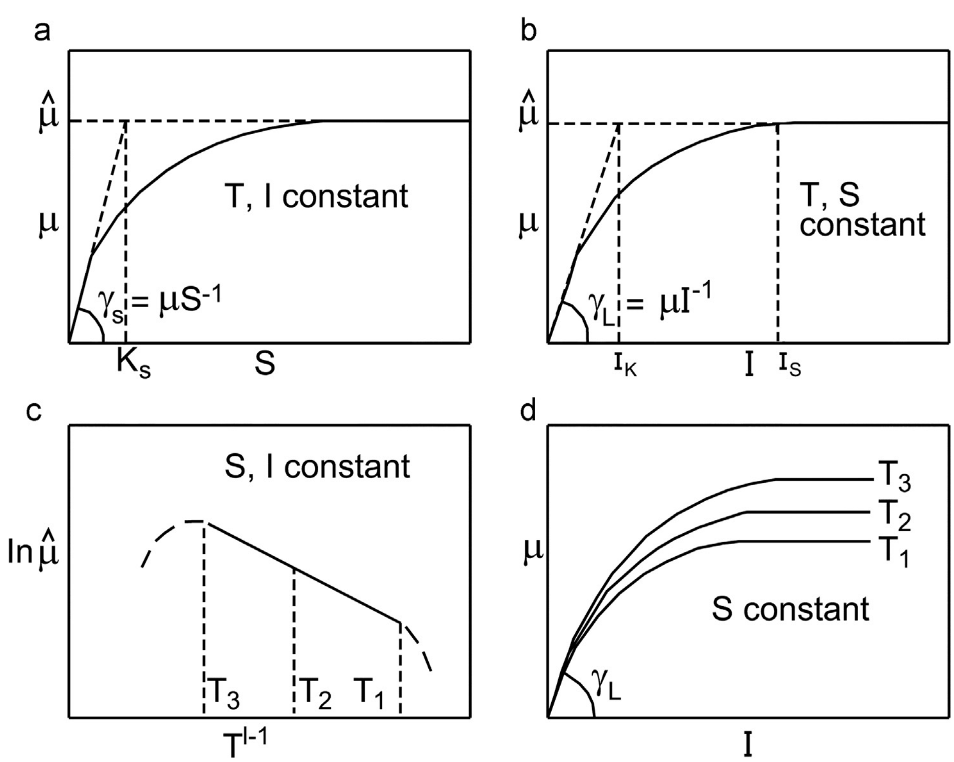


Fig. 14. The general functionality between algal growth rate (μ) and environmental parameters is conveyed through four different scenarios shown in the above graphs representing the following: (a) nutrients (S) are increased while temperature (T) and light intensity (I) held constant, (b) I is increased while T and S are held constant, (c) T is increased while S and I are kept constant, (d) S held constant and I being changed for various temperatures (Adapted from [155]).

Fig. 14c shows the equation applicable over a temperature range (~10–40 °C) where most of the algae grow. In Fig. 14c, the In $\hat{\mu}$ to T' relation is shown as being linear between T_1 and T_3 only. For algal culture, all nutrients are supplied in excess, so that the effect of both temperature and sunlight intensity on growth rate are as shown in Fig. 14d.

8. Algae harvesting

Algae are typically cultured in highly diluted water suspensions. Separating algae from its culturing media is known as harvesting. Harvesting also allows the drying, and further processing of microalgae to obtain the desired products. Due to the microscopic size of the microalgae and the diluted nature of their culture, algae harvesting is a cumbersome process. The most common harvesting processes are flocculation, micro screening and centrifugation. These processes must be energy-efficient and relatively inexpensive in order for the overall process to be viable. Therefore, selecting easy-to harvest strains of microalgae is very important.

However, a major difficulty to achieve significant quantities of microalgal biomass is its relatively high estimated cost of about \$10/kg, with a harvesting cost amounting to about 25% of the total production cost. As a reference, canola oil can be produced at about \$1/L. Hence, the success of microalgal biofuels depends very much on an enhanced process economy.

Microalgal cells are negatively charged. This is the result of the adsorption of ions originating from organic matter and due to the dissociation or ionization of surface functional groups [164]. These negative charges prevent self-aggregation as in the case of colloidal particles. Disrupting charges may lead to successful microalgal harvesting. This can be accomplished by adding inorganic flocculants such as $Al_2(SO_4)_3$ (aluminum sulfate), $FeCl_3$ (ferric chloride), $Fe_2(SO4)_3$ or organic/polyelectrolyte flocculants. Knuckey et al. [165] used Fe^{3+} flocculants with induced pH to harvest various kinds of algae and achieved harvesting efficiencies of 80%. Biodegradable organic flocculants, such as chitosan, are produced from natural sources that do not contaminate the microalgal biomass [166]. Thus, the resulting biomass can be used in food, feed and nutraceuticals. The most effective flocculants for the recovery of microalgae are cationic flocculants [167]. On the other hand, anionic and nonionic polyelectrolytes have been shown to fail to flocculate microalgae. This can be explained given the repulsion as a result of existing charges or the insufficient distance to bridge particles.

Levin (1961) developed a highly efficient froth flotation procedure for harvesting algae from diluted suspensions. The method does not depend upon the addition of floating promoters. Harvesting is carried out in a long column containing the feed solution which is aerated from below. A stable column of foam is produced and the microalgae are harvested from a side arm near the top of the column. Harvesting involves "biofloculation" in which the algae spontaneously flocculate and sediment in the settling chambers/ponds. This is a well-known natural process which has been demonstrated in waste grown algae [168].

Lee et al. [169] introduced "Electroflocculation" for harvesting marine microalgae species. These authors implemented this technique in an electroflocculation container equipped with electrode plates and a direct current power supply. Vandamme et al. [170] proposed "electro-coagulation–flocculation" (ECF) as a method for harvesting a freshwater *C. vulgaris* and a marine *Phaeodactylum tricornutum*. Under optimal conditions, power consumption of the ECF process was around 2 kWh/kg of microalgal biomass harvested using *C. vulgaris* and ca. 0.3 kWh/kg for *Phaeodactylum tricornutum*. Thus, it has been proven that electroflocculation can be implemented with a modest capital investment and limited use of electricity.

Liu et al. [171] performed harvesting algae species experiments using flotation techniques. The Liu et al. [171] flotation technique used is a flotation acrylic column with an inner diameter of 3 cm. A lipped side arm placed at 5 cm from the column top served to discharge foam while a 10–16 μm pore size injector at the column bottom was added to spurge the gas. A side arm with stop clock was used for sampling. Cationic *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (CTAB) and anionic sodium dodecylsulfate (SDS) were employed as collectors, respectively. Measured amounts of stock solution of collector and algal cells were added to a 500-mL volumetric stirred flask. The pH was adjusted with 0.5 N NaOH and 0.5 N HNO_3. A steady flow rate of air was inserted before a 200 mL

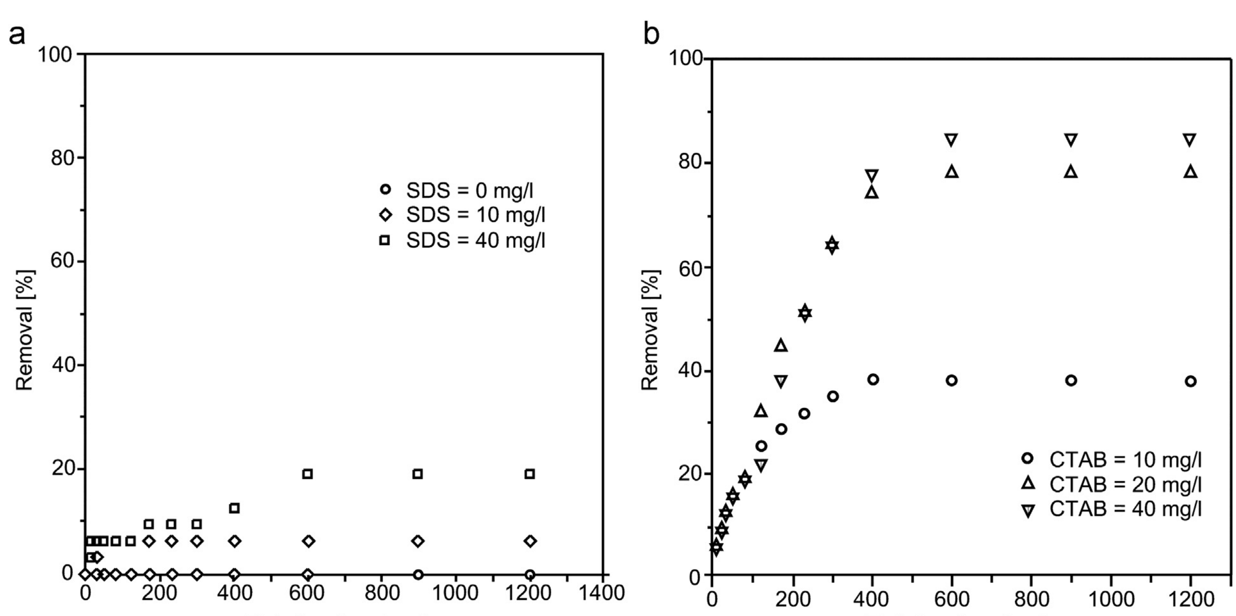


Fig. 15. Harvesting of *Chlorella* sp. using (a) SDS and (b) CTAB as collector. I=0.05 M NaNO3, initial cell concentration=6.8 × 10^5 cell/mL, pH=8.0 ± 0.1, air flow rate=114 mL/min (Adapted from [171]).

suspension was transferred to the flotation column. The duration of flotation was 20 min for all runs. Samples were taken at certain time intervals and the algae concentrations were measured.

Fig. 15 shows poor cell removal for the case of SDS while compared with that of CTAB at same operating conditions. Poor cell removal was achieved when the SDS used was 10 mg/L. The cell removal however increases with the raising of SDS concentration. Furthermore, removal efficiency augments with CTAB concentration, reaching 86% at the concentration of 40 mg/L. It is considered that collector ions at the air–liquid interface may become adsorbed on the solid surface and thereby increase the solid particle hydrophobicity. This may provide electric interactions between gas bubbles and solid particles. It is speculated that the electrostatic interaction between collector and cell surfaces plays a key role in the separation process. It was observed in the current work that both CTAB and SDS are good frothers with fine and stable air bubbles being generated. Nevertheless, the adsorption of collector ions onto cell surfaces was a prerequisite for effective separation. Contrary to SDS, CTAB was favorably adsorbed onto the cell surfaces through electrostatic interaction under neutral to slightly alkaline pH conditions. Cell surfaces then became more hydrophobic and were easier to separate from water.

Algae which have a diameter of more than 5 μm in size have thicker cell walls and can be separated by gravitational forces using centrifugation. Molina et al. [172] tested this technique in the laboratory by developing a prototype pond system with effluent carrying 500–1000 g of microalgae showing that about 80–90% microalgae can be removed within 2–5 min. These authors mentioned that this method is more effective than previous ones. However, it is time consuming and costly. Centrifugation is, in this context, considered a secondary harvesting technique which follows after filtering from a 1–2 g/L to 100–200 g/L paste [173]. Hawkins et al. used sum of chlrophyl-a and pheophytin-a to understand the food quality of microalgae after harvested followed by centrifugation [174].

9. Lipid extraction and analysis

Lipids are one of the main components of microalgae. Depending on the species and growth conditions, microalgae contain around 2–60% lipids of the total cell dry weight. Lipid oils derived from microalgae have been the focus of considerable interest because these oils contain fatty acid and triglyceride compounds that can be esterified into alcohol esters. The resulting so-called ester fuels have proven suitable for blending with diesel stocks up to 30% without affecting engine performance [175]. Some investigators have also successfully used them as neat fuels [176].

Table 14
Lipid productivity (mg/L d) using different microalgae species.

Microalgae species	Lipid productivity (mg/L d)	References
Chaetoceros calcitrans	17.6	[178]
Chaetoceros muelleri	21.8	[178]
Chlorella sorokiniana	44.7	[178]
Chlorella vulgaris	36.9	[178]
Chlorococcum sp.	53.7	[178]
Ellipsoidion sp.	47.3	[178]
Nannochloris sp.	76.5	[179]
Nannochloropsis sp.	61.0	[178]
Neochloris oleoabundans	125	[178]
Pavlova lutheri	50.2	[178]
Pavlova salina	49.4	[178]
Phaeodactylum tricornutum	44.8	[178]
Porphyridium cruentum	34.8	[178]
Tetraselmis sp.	22.7	[181]
Isochrysis sp.	21.1	[168]
Chlorella vulgaris	14.7	[180]
Chlorella emersonii	50.0	[182]
Chlorella protothecoides	1214	[183]

Microalgae are unique, photosynthetically driven chemical factories. More than 40 years of research into microalgae has demonstrated that these microorganisms can produce a diverse array of chemicals and hydrocarbon fuels. Fatty acid or lipid synthesis when using microalgae is an oxygen dependent process, producing mostly fatty acids with 16 carbons to 22 carbons chain length [177]. Factors such as (a) nutrient concentration, (b) salinity, (c) light intensity,(d) CO_2 concentration and (e) temperature all influence the lipid content and distribution of the cells.

In algal cells, nonpolar lipids (triglycerides) are carbon compounds which provide means to store energy. On the other hand, phospholipids and glycolipid are polar lipids inside cells, whose function includes forming cell and chloroplast membranes [124]. Although polar lipids can be converted into biodiesel, traditional feedstocks are the non-polar triglycerides.

Thus, non-polar lipids are most desirable algal products. Lipid composition and productivity depend on growth conditions in the growth phase [175]. The total lipid content varies between species ranging from very low (4.5%) to very high (80%) [176]. Lipid productivity can vary for different microalgae cultures as reported in Table 14.

9.1. Lipid extraction

It is evident that extracting the lipid from microalgae is a significant challenge for biofuel production with relatively low energy-intensive and cost. The development of the method for the extraction and purification of the lipids from dry biomass is critical for diesel production from microalgae. However, the exact conditions to accomplish this have not yet being fully specified [1,184].

Lipid extraction from biological material is performed by chemical means, physical means or a combination of the two. Cell disruption is often necessary for recovering intracellular lipid and sugar from microalgae, for biodiesel and ethanol production. For large scale lipid extraction from microalgae, the process is usually accomplished with mechanical cell disruption followed by chemical solvent extraction. There are also other mechanical processes reported in the literature such as freezing, osmotic shock, sonication, high pressure homogenization, bead milling and chemical processes such as alkali and organic solvent extraction [1,184–186].

9.1.1. Mechanical extraction

Rapid stirring of the thickened suspension of microalgae or other organisms in the mills is required for the extraction of lipids by cell disruption. Cell disruption may occur by the crushing action of the glass beads while colliding with the suspended cells. Hopkins [187] reported that a large number of tiny sizes glass beads or ceramic beads are vigorously agitated by shaking or stirring. The basic setup of a bead mill is a jacketed grinding chamber with a rotating shaft through its center. The shaft is fitted with discs that impart kinetic energy to the small beads in the chamber, forcing them to collide with each other. The beads are retained in a grinding chamber by a sieve or an axial slot smaller than the bead size. The beads are accelerated in a radial direction, forming stream layers of different velocity and creating high-shear forces. An external pump feeds the suspension into the grinding chamber.

Ultrasound is a process now widely used for microcell disruption like that used for microalgae and other microbial processes. Under the proper conditions, this device generates intense sonic pressure waves in the liquid media with formation of small bubbles. These small bubbles create cavitation by growing,

collapsing and violently generating huge shock waves of energy breaking cell membrane walls [188]. Converti et al. [189] described the use of ultrasound (mod. UP100H, Hielscher, Teltow, Germany). The authors combined the use of ultrasound with chloroform/methanol and this allowed the complete extraction of the microalgae fatty components. Cerón et al. [190] extracted lutein from the microalga *Scenedesmus almeriensis*. They tested three cell disruption methods, such as (a) mortar and pestle (125 mL volume), (b) bead mill (2 L volume and a rotation speed of 120 rpm, with ceramic beads of 28 mm diameter), and (c) ultrasound (Pselecta Ultrasons unit) and (d) a combination between them. Their results demonstrate that cell disruption is necessary. They showed that the best option among the treatments tested with regard to industrial applications was the use of bead mill with alumina in a 1:1 w/w as disintegrating agent for 5 min.

Neto et al. [191] studied lipid extraction by using a sonication bath pretreatment cell disruption step, followed by vortex mixing and n-hexane solvent extraction. These authors proved that this method allowed a higher lipid extraction from algal biomass than conventional hexane solvent extraction. Authors hypothesized that ultrasonic-assisted lipid extraction reduces both the extraction time and the solvent consumption, as a result of greater penetration of solvents into the cell structure [192].

Like other microbial cells, microalgae can be frozen in liquid nitrogen or freeze dryer to harden it. These hard cells are brittle in nature and can be easily crushed using a mortar and pestle. Ice crystals at these low temperatures are abrasive. Gouveia et al. [193] extracted lipids using mechanical crushing by freezing microalgae followed by supercritical CO_2 extraction. They manually crushed microalgae and mixed it with dry ice, moderately. Following this, they crushed the mixture completely with a disk vibratory mill (NV-TEMA, Labor-Scheibenschwingmuhle, type T100, 0.75 kW, 1000 V/min).

Zheng et al. [194] extracted lipids by using *C. vulgaris* sp. They compared various lipid extraction processes such as (a) quartz sand grinding under wet conditions, (b) quartz sand grinding under dehydrated condition, (c) grinding in liquid nitrogen, (d) ultrasonication, (e) bead milling, (f) enzymatic lysis by snailase, (g) enzymatic lysis by lysozyme, (h) enzymatic lysis by cellulose, and (i) microwaves. The shortest disruption time was 2 min using grinding in liquid nitrogen.

9.1.2. Chemical/solvent extraction

The solvent extraction of oil typically follows the mechanical disruption of algae cells and can be performed using a two solvent system (non-polar and polar). At the lab scale, where often more mechanical disruption proceeds, this can be done by the method developed by Bligh and Dyer [195]. This method uses a chloroform/methanol/water extraction procedure to obtain lipids from the cells. The non-polar (chloroform) and polar (methanol) solvents are used to extract the lipid fractions from the cells. Solvent extractions have the main advantage of obtaining generally high recoveries of lipids which can be further refined or used in a crude form.

Dote et al. [196] assessed the effectiveness of liquefaction for converting the algal cells of *B. braunii* into liquid fuels. Algal cells (~30 g) were charged to the autoclave with or without a catalyst (sodium carbonate). Distilled water (20 mL) was added, because the quantity of algal cells was too small for adequate stirring. After purging with nitrogen, the autoclave was charged with nitrogen at 2 MPa and then sealed and heated by an electric furnace.

Supercritical CO_2can also be used as a solvent to extract lipids from microalgae. Supercritical $CO_{2,}$ allows CO_2 to retain some of the liquid and gas. Supercritical fluid extraction is a ''natural and green'' approach for product extraction. This method has received increased attention as an important alternative to conventional separation methods. It is simpler, faster and more efficient. Furthermore, it avoids the consumption of large amounts of organic solvents, which are often expensive and potentially harmful. Supercritical CO_2 presents some unique characteristics as a solvent, since it is not toxic [197]. Therefore, it can be a good candidate for lipid extraction from the microalgae *C. vulgaris*, as successfully demonstrated by Mendes et al. [198,199] and Gouviea et al. [193]. Mendes et al. [198] reported that mechanically crushed microalgae give higher amounts of extraction yields using supercritical CO_2than those using normal extraction methods.

9.2. Lipid analysis

Nile Red is a fluorophore which becomes fluorescent when exposed to a specific wavelength of light in non-polar environments. This is the case for lipid droplets in algal cells. Nile red dye was used by Cooksey et al. [200] to determine non polar lipid content in algal cells. The authors in this study used cell cultures that were stained with Nile Red in acetone using a concentration of 1 μg/mL cell cultures. The average peak fluorescence was measured using a spectrofluorometer to gravimetrically determine total lipids for *Amphora coffeaeformis*, *Navicula* sp., *Tropidoneis* sp., and *Chlorella* sp.

There is a large variety of analytical techniques for the detection, the characterization and the quantification of fatty acids (FAs) in biodiesel. The two most common analytical techniques for analyzing fatty acids and triglycerides are gas chromatography(GC) and high performance liquid chromatography (HPLC). The comparison of these two techniques should focus not only on the chromatographic analysis but also on the sample preparation. Although the application of HPLC to fatty acid analysis has increased over the last decade, GC is still the most widely used technique [201]. This well-established procedure coupled with gas chromatography-flame ionization detection (GC-FID) is very efficient and rapid when complex mixtures with a broad molecular range are analyzed [202]. Fatty acids are suitable for the separation, the quantification and the analysis by GC without any need of derivatization, as demonstrated by James and Martin [203]. However, derivatization of fatty acids for GC analysis can be performed to increase the volatility of the substances, to improve separation, and to reduce tailings. Recent developments in the technology of GC columns with bonded phases offer unique separation capabilities with little phase bleeding. However, in cases where higher sensitivity is required then derivatization may be used. In recent times, GC has been used for the separation and analysis of geometric and positional isomers. Furthermore, GC can be complemented with mass spectrometry (MS) detection. MS has been demonstrated to be a powerful technique for diagnostic fragmentation of saturated and unsaturated FAs, as well as for the analysis of branching positions in FAs [204].

Otsuka and Morimura [205] used synchronously grown cultures of *Chlorella ellipsoidea* to show the change in lipid composition over the stages of cell growth. A methanol/ethanol/ether lipid extractions were followed by chromatography using a silicic acid column. Polar and nonpolar lipids were separated and quantification of the individual fatty acid methyl esters fractions was obtained. These authors analyzed cell growth at various culture stages. The resulting fatty acid profile reported by the growth stage showed a change in the relative distribution of polar and non-polar fatty acids at successive periods in the cell growth.

Depending on the species, microalgae produced many different kinds of lipids, hydrocarbons and other complex oils [206]. It was found that oleic acid (C18:1) and palmitic acid (C16:1) are the most abundant fatty acids in algae. Oleic acid is consumed during cell division regardless of whether the cells are under light or dark

conditions, suggesting that oleic acid in triglycerides are a major source of energy during cell division.

Manipulation of the fatty acid composition of microalgae may be accomplished by changing the culture conditions. Gurkert and Cooksey [124] performed experiments that showed how the fatty acid profiles of *Chlorella* sp. changes with pH. pH increases as a result of CO_2 consumption. The intent of these experiments was to show how the fatty acids profile changed with nitrogen deficiency. It is commonly accepted that nitrogen deficiency increases non-polar lipid production in microalgae, hence making the algae more useful for enhanced CO_2 mitigation and biofuel production.

On the other hand, lipid accumulation generally happens because the cell growth cycle is inhibited at times and cell division is delayed as a result. During this time, the cells continue to make and store lipids in anticipation of division. Thus, the neutral lipid content increases. The fatty acid composition of two microalgae species relative to the one of sunflower oil were studied by Hill et al. [207] as reported in Fig. 16.

Regarding algae species and the sunflower oil content, a main feature was the large amount of linoleic acid (C18:2) contained. In this respect, microalgae species show a higher unsaturation degree (C16:3, C16:3, C20+:1+) in the longer chain fatty acids, and in shorter chain fatty acids (C14:0, C16:0) [207]. As a result, and overall, a much greater variety of acids is contained by microalgae species.

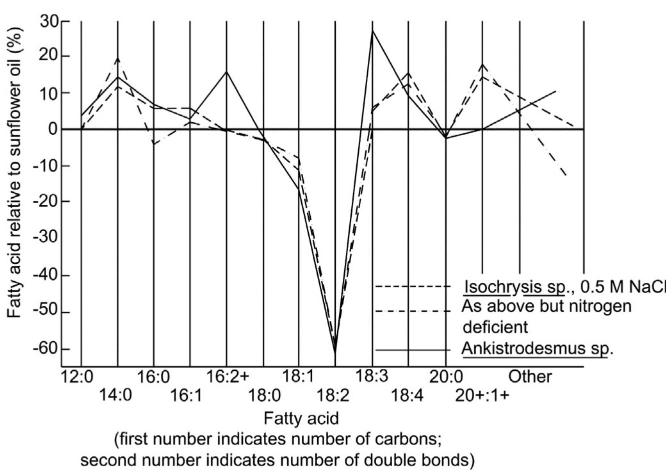


Fig. 16. Algal lipid profiles relative to sunflower oil (Adapted from [207]).

10. Biofuel production

Due to the increase of fossil fuel costs and as a result of the increased impact of green house gases, renewable energy is the best choice. Carbon neutral renewable liquid fuels are needed to eventually reduce the use of petroleum derived fuels that contribute to global warming [184]. There are in this respect, different ways to produce biofuel from microalgal biomass. These processes can be classified into chemical, biochemical and thermochemical conversion processes as shown in Fig. 17.

10.1. Chemical conversion

10.1.1. Transesterification

The basic chemistry of biodiesel production is relatively simple and mainly occurs via transesterification, in which a triglyceride (lipid) reacts with a mono-alcohol (most commonly ethanol or methanol) in the presence of a catalyst [1,208]. In general, there are three categories of catalysts that can be used for biodiesel production: alkalis, acids, and enzymes. Biodiesel is a low-emission diesel, a fuel substitute manufactured from renewable resources and waste lipids.

The most common way to produce biodiesel is through transesterification, especially alkali-catalyzed transesterification. As a result, the conversion of the methyl esters of the fatty acids (FAME) yields glycerol (refer to Fig. 18). When the raw materials

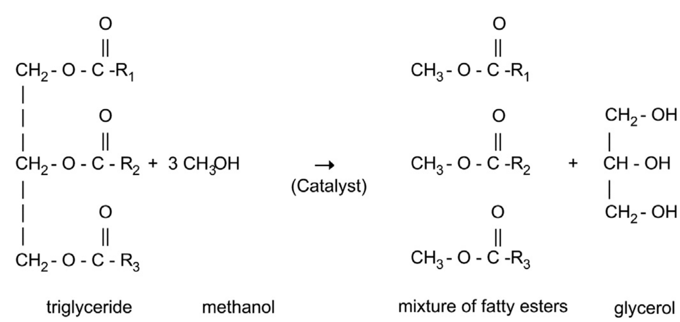


Fig. 18. Transesterification of triglyceride to fatty acid esters.

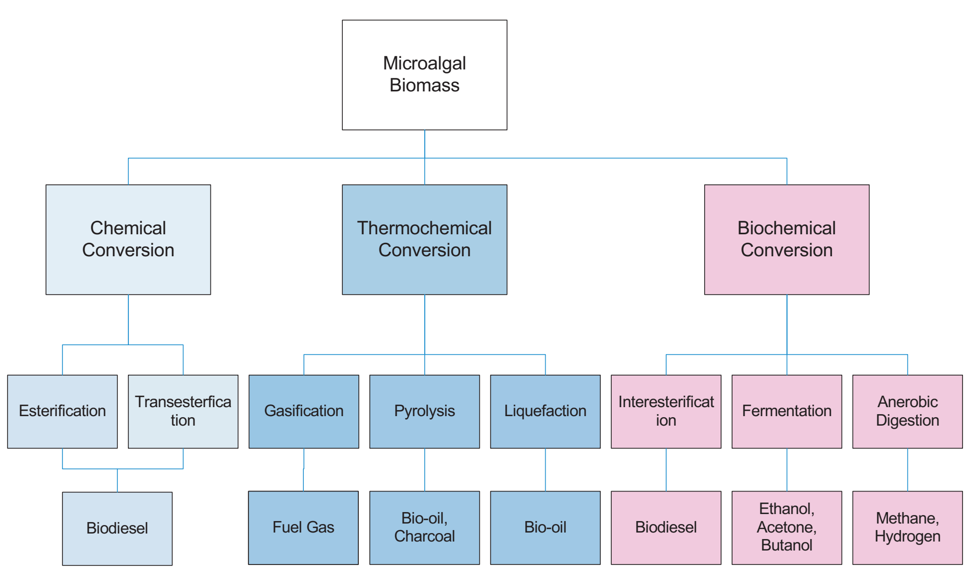


Fig. 17. Biofuel production from microalgal biomass using chemical, thermochemical and biochemical conversion processes (adapted from [95]).

Table 15
Comparison of sources of biodiesel [1].

Crop	Oil yield (L/ha)	Land area needed (Mha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

(oils or fats) have a high percentage of free fatty acids or water, the alkali catalyst reacts with the free fatty acids forming soaps. The water present can hydrolyze the triglycerides into diglycerides, forming more free fatty acids. Both described reactions are undesirable and reduce the yield of the biodiesel product. In this situation, the acidic materials should be pretreated to inhibit the saponification reaction [209].

Biodiesel has received considerable attention in recent years, as it is a biodegradable, renewable and nontoxic fuel [209]. Biodiesel is an alternative environmentally friendly liquid fuel that can be used in any diesel engine without modification. Biodiesel can also be used in regular combustion engines by mixing it with a regular derived fossil fuel at the adequate ratios [209].

Chemically, biodiesel is a mixture of methyl esters with long-chain fatty acids and is typically made from nontoxic, biological resources. When compared to emissions from fossil fuel derived diesel, emissions produced from biodiesel in combustion engines are reduced as follows: 100% SO_2, 48% CO, 47% particulate matter, 67% CO_2 [210]. Thus, the use of biodiesel leads to zero net CO_2, no sulfur and significantly reduced permanent gas emissions [211,212]. Although the environmental considerations for biodiesel are particularly favorable, its major drawback is the potential increase of NO_x emissions over petroleum derived diesels [213].

Biodiesels can be produced from different plant oils. There are, however, concerns about the sustainability of this practice due to food scarcity and limited lands available for cultivation. Microalgae have recently received significant attention as an alternative biomass source for the production of renewable energy. Main characteristics of microalgae make it stand apart from other sources due to its higher yield per year per unit area and higher lipid contents. Microalgae can be grown in unproductive land without affecting the crop cultivation system.

Table 15 shows the comparison of the sources of biodiesel in the United States as reported by Chisti [1]. Among all crop sources, diesel from palm oil cultivation can meet 50% of transportation oil fuel demand. This can be met by using 24% of the existing cropping area in US. Thus on this basis, oil from crops cannot significantly contribute to the use, reduction or replacement of fossil fuels. On the other hand, as shown in the Table 15, microalgae appear to be the only source of biodiesel than can produce major quantities of oil. In the USA, this could be accomplished with 1.1–2.2% of cropping area leading to 50% reduction in the usage of derived fossil fuels. Microlgae also can be cultivated in infertile marginal areas and save in this manner valuable fertile lands.

Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some microalgae exceeds 80% of the dry weight of the algae biomass. The use of algae as energy crops has the potential, due to their easy adaptability to growth conditions, of growing either in fresh or marine waters. Furthermore, and given that two-thirds of the earth's surface is covered with lakes and oceans, thus algae can become a truly renewable resource of great potential.

Current biodiesel manufacturing processes primarily employ transesterification as described earlier using primarily NaOH as a catalyst. This catalyst is corrosive to the equipment and also reacts with free fatty acids (FFA), forming foam and water as by-products. Water also helps breakdown triglycerides and produces more free fatty acids, increasing foam formation. An approach to reduce saponification is to use homogeneous sulfuric acid as a catalyst in order to convert free fatty acids first, followed by water removal and then by the subsequent conversion of tri and diglyceride to biofuel using NaOH as a catalyst [214].

In the last 15 years, many industrial processes have shifted towards the use of a solid acid catalyst instead to using liquid acid catalyst [215]. Kiss et al. [214] investigated a solid acid catalyst for removing free fatty acids which avoids the production of water. The goal was to enhance tri and diglyceride conversion to biodiesel without foaming (saponification) or at least to significantly reduce foam formation. These authors investigated solid catalysts with the aim of providing them with three important characteristics. First, the catalyst should be very active and selective in circumventing water formation, which likely renders the process uneconomical. Second, catalysts should be water-tolerant and stable at relatively high temperatures. Finally, catalysts should be made of an inexpensive material that is also readily available at an industrial scale. They tested different various solid acids as follows: (a) zeolites, (b) ion-exchange resins and (c) mixed metal oxide. They used these catalysts for the esterification of decanoic acids (free fatty acid) with 2-ethylhexanol, 1-propanol and methanol at 130–180 °C. They found that the most promising catalyst was sulphated zirconia based on its stability characteristics.

10.1.2. Esterification

The esterification process is a reversible reaction where free fatty acids (FFA) are converted to alkyl esters via acid catalysis as shown in Fig. 19 (HCl or H_2SO_4). The simultaneous application of esterification and transesterification reactions via acid catalysis reduces saponification achieving the almost complete conversion of biodiesel.

10.1.2.1. Base catalysis. Base-or alkali-catalyzed reactions typically using sodium hydroxide or potassium hydroxide are most common when oils are neutral or mostly free of fatty acids. Homogeneous base catalysts like sodium hydroxide, sodium methooxide or potassium hydroxide are used in the production of biodiesel primarily due to their low cost and high reaction rates. They can be used at low operating temperatures typically between 45 and 55 °C. The major drawback of these catalysts is their difficult recovery and highly corrosive nature [216]. Among the most available homogeneous catalysts used in biodiesel production, potassium hydroxide yielded better separation performance for oil feedstock containing FFAs. Leung and Guo [217] also noted that the potassium soap and glycerol formed during the reaction were much easier to remove than the other homogeneous catalysts. This is due to the fact that softer potassium soap is formed remaining unsettled in the glycerol layer. This imparts a lower viscosity to the glycerol solution making it easier to remove. Even though homogeneous catalyzed biodiesel production processes are relatively rapid and show high conversions with minimal side reactions, they are still not very cost-competitive with respect to petro-diesel ones. This is due to the following: (a) the catalyst cannot be recovered and must be neutralized at the end of the reaction, (b) there is a limited use of continuous processing methodologies and

$$\underset{RCOOH}{FFA} + \underset{R'OH}{Alcohol} \overset{Acid\ Catalyst}{\leftrightarrow} \underset{RCOOR'}{Alkyl\ Ester} + \underset{H_2O}{Water}$$

Fig. 19. Acid-catalyzed esterification

(c) the processes are very sensitive to the presence of water and FFAs. Consequently, there is a need for high quality feedstocks (e.g., virgin or refined VOs). This is required to avoid undesired side reactions such as hydrolysis and saponification or additional reaction treatment steps to convert the FFAs.

On the other hand, heterogeneous solid base catalysts are advantageous due to their ease to recovery and their potential for continuous processing and regeneration. Solid catalysts can be reused and this leads to lower production cost [218]. Liu and Goodwin [219] described solid base catalysts like ETS-10 (Na, K), calcium oxides, quaternary ammonium silica gels and other anionic exchange resins. Zeolites faujasite NaX and Titanosilicate Structure-10 (ETS-10) are predominantly alkali–cation-exchanged zeolite type materials. The basicity of zeolites NaX and ETS-10 can be enhanced by ion exchange with higher electropositive metals like K and Cs using conventional techniques. Sodium oxide clusters in the faujasite zeolites were occluded in zeolite cages via decomposition of impregnated sodium acetate or sodium azide to further increase their basicity [220].

A range of catalysts including (a) Mg/Al hydrotalcites, (b) alkali nitrate and alkali carbonate-loaded Al_2O_3, (c) polymer resins, (d) tin sulfide and zirconia oxides and (e) tungstated-zirconia have also been reported [220]. Most of these catalysts lose activity while being recycled and/or require pretreatment of the feedstock to remove the FFAs and water [220].

10.1.2.2. Acid catalysis. Acid catalysts are very effective in transesterification when the oil contains a high amount of FFAs. Acid catalysts convert FFAs to esters or biodiesel through esterification and simultaneously transesterification. This is achieved at a relatively higher temperature of around 100 °C. Lotero et al. [221] reported that acid-catalyzed transesterification occurs in four intermediate reversible steps, including (1) protonation of the carbonyl group with acid catalyst making the adjacent carbon atoms more prone to nucleophilic attack, (2) protonation of the alcohol group, (3) proton migration, and (4) breakdown of the intermediate species freeing the alkyl ester from the intermediate diacylglycerols (DAG). This DAG is then subject to further breakdown by repeating the series of intermediate reactions twice.

Acid catalyzed reactions were first developed using homogenous catalysts such as sulfuric acid, hydrochloric acid, BF3 (boron trifluoride), phosphoric acid and sulfonic acids [222]. Liu et al. [222] also reported that these reactions are initially controlled by mass transfer due to the low solubility of alcohols and reagents. Following this, a kinetically controlled reaction is established with rapid product formation. Finally, a near equilibrium reaction regime takes hold with a dramatically reduced rate of FFA product formation.

Recently heterogeneous solid catalysts are becoming more attractive due to their good adsorption properties, easier handling and regeneration capabilities, low cost of operation and adequate use in a continuous process system. So far, heterogeneous solid catalysts including zeolites, silica molecular sieves doped with metals (e.g., aluminum, titanium), sulfated zirconia, tungstated zircon, sulfonated tin oxide, Amberlyst-15,75 and Naphion NR50 have been proposed [221].

10.2. Thermochemical conversion

Thermo-chemical conversion (TCC) is a high-temperature reforming process that breaks apart the bonds of organic matter and restructures its intermediates chemical species into char, synthesis gas and highly oxygenated bio-oil. TCC can be a major consumer of manure's organic components, extracting valuable available energy. TCC processing has a number of other benefits and advantages. These are (1) a small footprint; (2) an efficient nutrient recovery; (3) no fugitive gas emissions; (4) short processing time in the order of minutes; (5) capability of handling a variety feedstocks and blends; and (6) high-temperature elimination of pathogens and pharmaceutically active compounds [223,224]. After conversion, TCC processing leaves minor residues such as ash requiring disposal. Ash can be used as a fertilizer reducing disposal fees associated with fuel, dumping and transportation costs.

10.2.1. Gasification

Biomass gasification is a thermochemical conversion process in which incomplete combustion of biomass results in the production of combustible gases, called producer gases. Producer gas consists of a mixture of carbon monoxide (CO), hydrogen (H_2) and traces of methane (CH_4). The product gas has low calorific value (about 4–6 MJ/N m^3), and it can be burned directly or used as a fuel for gas engines and gas turbines. It can also be used as a feedstock in the production of chemicals (e.g. methanol) [225].

A novel energy production system using microalgae with nitrogen cycling combined with low temperature catalytic gasification of the microalgae has been proposed. Elliot has also developed a low temperature catalytic gasification of biomass process with high moisture content [226]. Biomass with high moisture is gasified directly to methane rich fuel gas without drying. In addition, nitrogen in the biomass is converted to ammonia during the reaction.

10.2.2. Pyrolysis

Pyrolysis is the thermal decomposition of materials in the absence of oxygen or when significantly less oxygen is present than required for complete combustion to obtain an array of solid, liquid, and gas products. The pyrolysis method has been used for the commercial production of a wide range of fuels, solvents, chemicals, and other products from biomass feedstocks. Pyrolytic oil may be used directly as a liquid fuel, added to petroleum refinery feedstocks, or catalytically upgraded into transport grade fuels. In all thermochemical conversion processes, pyrolysis plays a key role in the reaction kinetics and hence in reactor design while determining product distribution, composition, and properties [227].

Bio-oil comparable to fossil oil was obtained from microalgae [206]. The yield of bio-oil from pyrolysis of the samples increased with temperature whereas charcoal decreased with increasing pyrolysis temperature. The yield of the liquid product is highly excessive at temperatures between 625 and 725 K [228]. The feasibility of producing liquid fuel or bio-oil via pyrolysis or thermochemical liquefaction of microalgae has been demonstrated with a range of microalgae. Since algae usually have high moisture content, a drying process requires much heating energy. A novel energy production system using microalgae with nitrogen cycling combined with low temperature catalytic gasification of the microalgae has been proposed.

10.2.3. Liquefaction

Appell et al. [229] reported that a variety of biomasses such as agricultural and civic wastes could be converted, partially, into a heavy oil-like product by reaction with water and carbon monoxide/hydrogen in the presence of sodium carbonate. These processes require high temperatures and pressures. In the liquefaction process, biomass is converted to liquefied products through a complex sequence of physical structure and chemical changes. In first step, biomass is decomposed into small molecules. These small molecules are unstable and reactive, and can repolymerize into oily compounds with a wide range of molecular weight distributions. Liquefaction can be accomplished directly or indirectly. Direct liquefaction involves rapid pyrolysis to produce liquid

tars and oils and/or condensable organic vapors. Indirect liquefaction involves the use of catalysts to convert non-condensable, gaseous products of pyrolysis or gasification into liquid products. Alkali salts, such as sodium carbonate and potassium carbonate, can hydrolyze cellulose and hemicellulose, into smaller fragments. Liquefaction can be performed by using a stainless steel autoclave with mechanical mixing. A number of studies on the use of hydrothermal technology are for direct liquefaction of algal biomass have already had been published in the literature.

Minowa et al. [230] utilized direct hydrothermal liquefaction at 575 K and 10 MPa and used *Dunaliella tertiolecta* with a moisture content of 78.4 wt%,. They reported an oil yield of 37% (organic basis). The oil produced had a viscosity of 150–330 MPa s and a heating value of 36 MJ/kg. Liquefaction of *B. braunii*, a colony-forming microalga, with high moisture content was performed with or without sodium carbonate as a catalyst for conversion into liquid fuel and recovery of hydrocarbons. The oil was equivalent in quality to petroleum oil [206]. Hydrocarbon recovery was at a maximum (> 95%) at 575 K.

Liquefaction of algal cells by hexane extraction yielded 58% of its dry weight producing oil with good fluidity, 56 cpviscosity and 49 MJ/kg heating value. The yields of the primary oil obtained at 575 K and 475 K were 52.9% and 56.5% respectively. Furthermore, the heating value of the primary oil obtained at 575 K was much higher than that attained at475K: 47.5 MJ/kg for 575 K and 42.0 MJ/kg for 475 K. Given the low oil viscosity, the primary oil obtained at 575 K could be used as fuel oil [196].

Thus, it can be concluded that the liquefaction technique can be a net energy producer, with hydrothermal liquefaction being more effective for extraction of microalgal biodiesel than for supercritical carbon dioxide [87]. From these studies, it is reasonable to believe that, among the selected techniques, hydrothermal liquefaction is the most effective technological option for the production of biodiesel from algae.

10.3. Biochemical conversion

10.3.1. Interesterification

Recently, enzymatic interesterification has attracted attention for biodiesel production as it yields a highly pure product and enables easy separation from the by product, glycerol [231]. Interesterification reactions via enzyme catalysis are biologically significant pathways for the production of fatty acid esters. This may be accomplished efficiently with enzyme catalysis using lipases secreted from various organisms such as *Candida antarctica*. This reaction results in the production of triacetin and long-chain fatty acid methyl or ethyl esters, with no glycerol formed as in the case of esterification and transesterification with alcohols (Fig. 20). In addition, the production of biodiesel using a biocatalyst eliminates the drawbacks of the alkali process by making a product of very high purity with less or no downstream post-treatment operations required [232].

Enzymatic diesel production is possible using both extracellular and intracellular lipases. In both the cases, the enzyme is immobilized. This eliminates downstream separation and recycling operations. In these studies, either immobilized (extracellular) enzymes or immobilized whole cells (Intracellular enzymes) are used as catalysts. Both these processes are reported as highly efficient when compared with the use of free enzymes [233]. Thus, immobilization allows enzymes to be reused without separation. Furthermore, the operating temperature of the process can be reduced to50 °C. Intertransferification disadvantages include reaction inhibition which was observed when methanol was used and high enzyme cost [233,234].

$$\underset{TAG}{Triglyceride} + \underset{3CH_2CH_3(OC(O)CH_3)}{EthylAcetate} \overset{Lipase}{\leftrightarrow} \underset{3RCOOCH_2CH_3}{EthylEster} + \underset{C_3H_5(OC(O)CH_3)_3}{Triacetin}$$

Fig. 20. Interesterfication procedures using lipase catalyst.

10.3.1.1. Enzyme catalysis. Enzyme catalysts have become more attractive recently since they avoid soap formation. Without soap, the post-biodiesel production process becomes much simpler. However, enzymes are less active, requiring longer reaction times. They are also expensive. To reduce the cost, researchers developed new biocatalysts. An example is the so-called cell biocatalysts where cells are immobilized within the biomass support particles. An advantage is that no purification is necessary in order to use these biocatalysts [232,235]. Modi et al. [231] mentioned that lipase-catalyzed systems can solve the problems associated with conventional homogeneous acid and homogenous base-catalyzed systems including glycerol recovery and inorganic salt removal.

In this regard, one could mention that enzymes typically need small amounts of water for activity. Thus, the difficulties concerning water which is formed by reacting alcohols in conventional systems can be circumvented with significant energy savings. Immobilized lipase enzymes hold even greater promise for similar reasons to the solid acid and base catalysts mentioned previously. These materials can be easily recovered and reused. Immobilized enzymes can also be affected by diffusional limitations and can be deactivated by alcohols such as methanol and ethanol. This activity decay can be inversely proportional to the number of carbons in the alcohol chain [236]. Hsu et al. [237] developed an immobilized lipase from *Pseudomonas cepacia* in a sol–gel polymer matrix that achieved more than 95% conversion of tallow (beef or mutton fat).

10.3.2. Fermentation

Fermentation is used commercially on a large scale in various countries to produce ethanol from sugar crops and starch crops. Corn (60–70% starch) is the dominant feedstock in the starch-to-bioethanol industry worldwide. However, algae can be used as raw material for ethanol production according to the following procedure: In the first step, the starch of microalgae is released from the cells with the aid of mechanical equipment or an enzyme. When the cells begin to degrade, *Saccharomyces cerevisiae* yeast is added to the biomass to begin fermentation. The product of fermentation is ethanol. The ethanol is drained from the tank and pumped to a holding tank to be fed to a distillation unit. Ethanol is produced with microalgal photosynthesis and intracellular anaerobic fermentation.

The chemical reaction involves enzymatic hydrolysis of sucrose followed by fermentation of simple sugars. Fermentation of sucrose is performed using commercial yeast such as *S. ceveresiae*. First, the invertase enzyme in the yeast catalyzes the hydrolysis of sucrose to convert it into glucose and fructose. Second, zymase, another enzyme also present in the yeast, converts the glucose and the fructose into ethanol. The gluco-amylase enzyme converts the starch into D-glucose. The enzymatic hydrolysis is then followed by fermentation, distillation and dehydration to yield anhydrous bioethanol.

A new and unique process has been developed when substrates such as carbohydrates are fermented by a consortium of bacteria; producing hydrogen and carbon dioxide. Hydrogen can be produced by algae under specific conditions. Three different ways to produce hydrogen have been proposed: direct and indirect photolysis, and ATP-driven hydrogen-production. Direct photolysis is possible when the resulting hydrogen and oxygen are continuously flushed away. Photosynthesis and water-splitting are coupled, resulting in the simultaneous production of hydrogen and oxygen. Direct hydrogen and oxygen direct contact results in major safety risks. There is also associated costs if one consider the

possibility of separation both species. Major factors affecting the cost of hydrogen production by microalgae are as follows: (a) the cost of the huge photobioreactor and (b) the cost of hydrogen storage facilities that also guarantee continuous hydrogen supply throughout the night or during cloudy periods of the day. Anaerobic hydrogen production proceeds with photo fermentation as well as without the presence of light. Anaerobic bacteria use organic substances as the sole source of electrons and energy, converting them into hydrogen.

The reactions involved in hydrogen production are rapid and these processes do not require solar radiation. Since they cannot utilize light energy, the decomposition of organic substrates is incomplete and decomposition of the acetic acid is not possible under anaerobic conditions. Nevertheless, these reactions are still suitable for the initial steps of wastewater treatment and hydrogen production followed by further waste treatment stages. A new fermentation process that converts valueless organic waste streams into hydrogen-rich gas has been developed by Hawkins et al. [174]. The process employs mixed microbial cultures readily available in nature, such as compost, anaerobic digester sludge, soil etc. to convert organic wastes into hydrogen-rich gas. The biodegradation efficiencies of the pollutants were examined by changing hydraulic retention time (HRT) as a main operating variable. An enriched culture of hydrogen producing bacteria such as *Clostridia* was obtained by heat treatment, pH control and HRT control of the treatment system. The bio-hydrogen fermentation technology could enhance the economic viability of many processes utilizing hydrogen as a fuel source or as raw materials.

Anaerobic fermentative microorganisms, such as cyanobacteria and algae are suitable in the biological production of hydrogen via hydrogenase due to reversible hydrogenases [239]. Hydrogen producing bacteria (*Clostridia*) were found to have growth rates about 5–10 times higher than that of methane producing bacteria. In a continuous flow bioreactor system, hydrogen production showed declining trends at the later stage of reactor operation. It is hypothesized that *Clostridia* may have gone through a degeneration process in which it loses its ability to produce hydrogen. Therefore, inoculating fresh mixed cultures may be a feasible way to maintain a sustainable hydrogen production. Based on this hypothesis, a two-stage anaerobic reactor has been proposed. The first-stage reactor is designed as a hydrogen producing reactor whereas the second-stage reactor will be employed to cultivate fresh seed culture to perpetually supply the first one [240].

10.3.3. Anaerobic digestion

Anaerobic digestion can be used as a source of renewable energy when it is fed with purpose-grown energy crops. This process produces a biogas, consisting of methane, carbon dioxide and traces of other ‘contaminant’ gases. This biogas can be used directly as cooking fuel, in combined heat and power gas engines or it can be upgraded to natural gas-quality biomethane. The Methane and the power produced in anaerobic digestion facilities can be used to replace energy derived from fossil fuels, and hence reduce emissions of greenhouse gases [15]. Nutrient-rich digestate is also produced as a by-product and can be used as a fertilizer. The digestion process begins with bacterial hydrolysis of the input materials to break down insoluble organic polymers, such as carbohydrates, and make them available to other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. Acetogenic bacteria then change these resulting organic acids into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. Finally, methanogens convert these products to methane and carbon dioxide [17].

A simplified generic chemical equation for the overall processes is $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$. Almost any organic material can be processed with anaerobic digestion; however, if biogas production is the aim, the level of digestible biomass is the key factor in its successful application. The more digestible biomass available, the higher the gas yields possible from the system. Digester processed dedicated energy crops can achieve high levels of degradation and biogas production. Digestion of the algae can be enhanced and the methane yield increased by physical or chemical pretreatment to break down cell walls and make the organic matter in the cells more accessible.

Moisture content of the feedstock is also an important factor to consider in the selection of the digestion system. Drying of food and yard waste is suitable for digestion in tunnel-like chambers. The wetter the material, the more suitable it will be to its handling with standard pumps instead of energy-intensive concrete pumps and physical means of movement. Another key consideration is the C (carbon) and N (nitrogen) ratio of the input material. This ratio is the balance of food a microbe requires to grow; the optimal C:N ratio is 20–30:1. Excess N can lead to ammonia inhibition of digestion [241]. One strategy to overcome this problem uses a ‘codigestion’ process, whereby other organic waste, which is higher in carbon and lower in nitrogen, is added to the algal waste.

There are numerous active biogas installations, from large-scale ones to small ones fed with straw and green plant fuel that serve a few farms; so far, however, algae have not been used as a fuel. Some macroalgal species like *Macrocystis pylifera*, and genera such as *Sargassum*, *Laminaria*, *Ascophyllum*, *Ulva*, *Cladophora*, *Chaetomorpha* and *Gracilaria*, have been explored as a potential methane source and good positive source of biogas using anaerobic digesters.

11. Conclusions

This comprehensive review covers the background of microalgae life cycles, looks at photo-bioreactor systems and discusses CO_2 capture. The following are the conclusion of this review article:

i) Photobioreactors could be effective in growing microalgae by using a favorable light source and reactor configuration.
ii) Bio-mitigation of CO_2 emissions with microalgae provides a complementary function that may moderate the cost of biofuels production. The use of waste CO_2 from power plants and other industries to enhance production of microalgae has been shown to be technically feasible, and hence, may be deployed to reduce production costs and for GHG emission control.
iii) Collection and concentration of microalgal biomass from cultivation systems contribute heavily to the operation cost of the overall process. Therefore, more efficient and economical harvesting technology should be developed to enhance the commercial viability of the microalgae biofuels industry.
iv) Biodiesel has great potential; however, the high cost and limited supply of organic oils prevent it from becoming a serious competitor for petroleum fuels. Using new and innovative techniques for cultivation, algae may allow the biodiesel production to achieve the price and scale of fabrication needed to compete with, or even replace, petroleum.

Acknowledgments

The authors would like to acknowledge the support provided by NSTIP-KACST for project NSTIP # 11-ENV1644-04. Authors also

like to thank King Fahd University of Petroleum & Minerals (KFUPM) and The University of Western Ontario. Authors would like to acknowledge Ms. F. de Lasa for her assistance on the preparation of this manuscript.

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